YEASTS IN FEEDING

F8,3Z01
N50
2467

CFTRI-MYSORE
2467
Yeasts in feedin

C.F.T.R.I.

467
asts, Vitamins,
east Cultures,
east Production,
east utilization,
animal feeds.

YEASTS IN FEEDING

Proceedings of the Symposium

November 8, 9, 10, 1948

THE HOTEL PFISTER

MILWAUKEE, WISCONSIN

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
LIBRARY
MYSORE.

Sponsored by

THE BREWERS' YEAST COUNCIL

THE YEAST INDUSTRY

THE QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES

THE ASSOCIATES, FOOD AND CONTAINER INSTITUTE, INC.

2467/G339

ACKNOWLEDGMENTS

The present brochure was made possible through the generosit the following companies: Jos. Schlitz Brewing Co., Pabst Brewing Red Star Yeast and Products Co., Standard Brands, Inc., Anhe Busch, Inc., Vico Products, Federal Yeast, National Yeast Comp Brewers Yeast Council, and Associates, Food and Container Insti Inc. When other publication resources had failed, representative several of these companies met and appropriated the funds necessary publication. Present at this meeting were Mr. James Veron, Anhe Busch, Inc., Mr. John Le Ware of Standard Brands, Mr. Alexa Frieden, Pabst Brewing Co., Mr. R. D. L. Wirth of Red Star Yeast Products Co., Mr. J. M. Leinen, Jos. Schlitz Brewing Co., Miss E Singruen, Brewers Yeast Council, Dr. Martin S. Peterson, Quar master Food and Container Institute, and Colonel Rohland A. Isker the Associates, Food and Container Institute. The general plan of brochure was developed at this meeting, and Colonel Isker and Peterson were appointed to arrange for the necessary revisions, edi and printing.

By unanimous agreement, Dr. Sadie Brenner was selected to edit papers, and the result of her careful and proficient labor is the pres brochure, *Yeasts in Feeding.*

Acknowledgments are also made to the various persons who ai in the planning and administration of the Symposium on *Yeasts in Fe ing,* held in Milwaukee in the fall of 1948. The success of the meeti was the product of their good planning and administration. Of th who aided in the planning, special credit is due Colonel Charles La rence, then commanding the QMF&CI, who initiated the idea, to Colonel J. S. Kujawski, presently commanding the QMF&CI, to George Gelman and Dr. Howard D. Lightbody, QMF&CI, to Mr. R. L. Wirth of the Red Star Yeast and Products Co., and to Miss E Singruen of the Brewers Yeast Council. Special appreciation is her expressed for the excellent address delivered before the dinner sess by Dr. C. A. Elvehjem, Dean of the Graduate College, University Wisconsin.

In the administration of the meetings and attendant activit acknowledgments are due to Mr. J. M. Leinen of the Jos. Schlitz Br ing Co., Colonel Rohland A. Isker, Dr. Emil Mrak, chairman, F Technology Department, the University of California, to Dr. Lightbo Dr. Harry Fevold, Miss Leona Wehrheim, Mr. Sydney G. Hambli Mr. Joe I. Abrams, of the QMF&CI, and to Miss Singruen, Dr. F Rudert, Red Star Yeast and Products Co., Mr. Walter Kessler, Pa Brewing Co., and to Mr. William W. Wadel, Public Relations Direc Blatz Brewing Co.

CFTRI-MYSORE
2467
Yeasts in feedin.
F8, 3201
N50

CONTENTS

Page

INTRODUCTION 1
C. G. King

NITROGEN COMPOUNDS OF YEAST 5
H. E. Carter

ENZYMES IN YEAST 9
M. J. Johnson

CARBOHYDRATES IN YEAST 15
Carl Neuberg

YEAST LIPIDS AND THEIR BIOLOGICAL SYNTHESIS 20
J. C. Hoogerheide

VITAMINS AND MINERALS OF YEAST 26
W. H. Peterson

VITAMIN AVAILABILITY 34
Helen T. Parsons

PROTEIN VALUES OF YEAST 40
Paul R. Cannon

COMPOSITION OF BREWERS' YEAST EXTRACTS 45
B. L. Maizel

EXPERIMENTATION WITH DRIED YEAST FOR USE IN FOODS 50
Sadie Brenner

EXPERIENCE IN USE OF YEAST IN COMMUNITY AND INDUSTRIAL FEEDING 59
Clive M. McCay

UTILIZATION OF YEAST IN CHICKEN AND TURKEY DIETS 64
James McGinnis

UTILIZATION OF YEAST IN LIVESTOCK AND SPECIALTY FEEDS 69
R. V. Boucher

OTHER PAPERS IN CONDENSED FORM

RELATION OF BASIC INVESTIGATIONS TO PRODUCTION AND UTILIZATION OF YEAST 73
M. A. Joslyn

Page

Basic Principles of Culturing and Production of Yeast 76
Lawrence Atkin

Brewers' Yeast-Recovery and Preparation For Use 78
W. L. Hegarty

Dry, Extracts, Pastes, Hydrolysates, Autolysates, Flavors 80
J. E. McClary

Commercial Production Practices For Growing Primary Yeast For Nutritional Purposes 82
Charles H. Keipper

Distillers' Dried Yeast 83
Gustave T. Reich

Food-Yeast Production From Wood-Processing Byproducts 85
Elwin E. Harris

Producing Yeast From Processing Wastes 87
W. D. Ramage and J. H. Thompson

Taxonomy and Morphology of Yeasts 89
E. M. Mrak and H. J. Phaff

Ecology and Distribution of Yeasts 92
H. J. Phaff and E. M. Mrak

Genetics and Hybridization in Saccharomyces 93
Carl C. Lindegren

Inheritance of Enzymatic Characters in Yeast 96
Catherine Roberts

The Experimental Control of Enzymatic Constitution in Yeast 97
S. Spiegelman

Nutrient Requirements of Yeast 99
M. A. Joslyn

The Role of Vitamins in the Life of Yeasts 101
Paul R. Burkholder

New Methods in Taxonomic Study and Maintenance of Yeasts 104
Lynferd J. Wickerham, Kermit A. Burton, and Richard J. Gill

Index 107

Introduction

Retired mess sergeants and our grandfathers may show expressions of doubt, amusement or amazement when vitamins and protein quality are mentioned in discussions of common foods. They would acknowledge at once, however, an appreciation of good fresh yeast in making a loaf of bread. They might even suspect the relationship of yeast to the development of foam and flavor in a barrel of cider.

The older nutrition scientists of the present generation have a respectful regard for yeast, too, because they remember the standard practice of putting 4% to 8% of dry yeast into nearly all experimental diets to provide "vitamin B." They soon discovered that when yeast was heated in a pressure cooker or autoclaved it lost something that rats needed for growth and for protection against an early onset of paralysis. The heated yeast still contained something that was essential to growth and health in rats and other animals. Optimistically, they called the first factor vitamin B_1 and the second, a more stable nutrient, vitamin B_2, and set out to isolate both of the active fractions.

There was no intimation from the experiments conducted twenty-five years ago, however, that the search to identify "vitamin B" would involve many completely different nutrients that came to light with continued study: B_6, choline, niacin, inositol, pantothenic acid, biotin, folic acid and vitamin B_{12}.

Nor is there any indication that the end of the trail is in sight. Instead, there is evidence already at hand that two additional vitamins required by chickens remain to be identified. There are indications that more than two new ones, perhaps as many as four, remain to be identified among the food requirements for health in mink.

Gradually, nutrition scientists learned to differentiate and measure by biological tests with rats, chickens, pigeons, dogs, monkeys, hamsters, pigs, molds and bacteria, the constituents of crude extracts from yeast that were essential for growth and health. Only at rare intervals could the investigators gain (in advance of isolation) even a glimpse of evidence that such tests might have value in the protection of human health, and hence serve to guide future developments in agriculture and in the food industry.

In parallel with the recognition of yeast as a remarkably good source of vitamins in the B-complex, there developed definite evidence that the protein content of yeast was also of good nutritive quality. This new

role of furnishing protein to supplement the protein in cereals gave to yeast a second basis for its rating as a "protective food." First in experimental diets and then in practical farm rations and in human food, yeast came to be recognized as an important source of protein with special merit in supplementing the protein supplied by other ingredients in bread.

The poultry and stock feed industries were quick to recognize the practical value of using yeast to provide generous sources of both protein and members of the vitamin B-complex, when fed along with grain, hay and other low-cost materials.

Irradiating yeast to form vitamin D_2 from otherwise inactive fatty material (ergosterol) in yeast gradually stimulated the use of vitamin D concentrates in a great variety of foodstuffs.

Pasteur's early and classic experiments on different kinds of yeast in French wines, followed by studies of the relation of yeast to developing desirable (and undesirable) types of fermentation, constitutes one of the most interesting chapters in the history of science. A by-product of these investigations, learning to protect milk supplies by pasteurization, constitutes one of the major technical developments in the food industry.

Commercial production and worldwide distribution of yeast was a logical development in meeting the needs of the baking industry, the brewing industry and in producing poultry and livestock feeds. In addition, enormous quantities continue to be used for the preparation of medicinal extracts and vitamin concentrates. The manufacture of yeast to be used directly as human food, other than as an ingredient of commodities such as bread, to provide protein of high quality and water-soluble vitamins in lieu of animal protein foods, has had a very limited development.

From a practical point of view, the acceptability of yeast in human food is limited by flavor and stability considerations. Most students of food economics or of food selection patterns do not anticipate a major development of yeast consumption as a separate commodity for the consumer—rather, one can look forward to increasing appreciation of its nutritive and economic value when incorporated into standard commodities such as bread, that has a high, almost universal acceptability combined with low cost.

The scope of scientific papers in the present volume gives evidence of the great range of information that has been developed by the yeast manufacturing industry and by scientific investigators in many related

fields of study such as exploratory research in biology, biochemistry, physiology, genetics, and food technology.

The fat content of yeast is ordinarily neglected in current discussions of food values. Some strains of yeast are characterized by a relatively high content of edible glyceride fat. However, much more attention has been given to the "complex" fats in yeast—especially to the sterols which serve as raw materials in the pharmaceutical industry and specifically as a precursor of vitamin D_2. The latter is readily activated by irradiation.

A sound basis for evaluating the nutritive quality of proteins in yeast has been established within the past year. Professor W. C. Rose at the University of Illinois has finally succeeded in establishing the amino acid requirement of human adults. Similar data are available for chickens and in lesser degree for other farm animals. This area of research, getting detailed information concerning the protein value of foods in terms of specific amino acids, is so complicated, however, by associated problems of vitamin intake, caloric expenditure, growth requirements, physiological disturbances imposed by illness, and general environmental factors, that it will remain a field of intensive investigation for many decades at least. Even with experimental animals, when genetic and environmental factors can be controlled to a high degree, it is difficult to arrive at satisfactory quantitative data. But in meeting human situations, it is far more difficult to reach satisfactory conclusions. The utilization of yeast in foods and feeds will become more efficient and more rational with each advance in basic nutritional knowledge.

A relatively recent advance that has greatly influenced peoples' thinking about the best ways of using yeast in human food has come from the evidence that heating or cooking yeast in typical food commodities contributes an advantage to its nutritive quality by making the vitamin and protein content more readily available. There is undoubtedly some destruction of specific nutrients as a result of heating, but increased availability of several vitamins more than compensates for the mild losses.

The degree to which yeast will find a place as a substitute for animal protein foods such as milk, cheese, eggs, meat and fish is difficult to predict at the present time. A reasonably conservative appraisal of world-wide experience thus far does not encourage us to anticipate major developments in that direction. Emergency periods incident to wartime conditions nevertheless may make developments of that nature a practical consideration; hence, it is well to anticipate the need for knowledge concerning its manufacture, processing and use in the event of such

emergencies. A good illustration of technological developments that may have critical significance during war-time should be cited in the case of staple dehydrated yeast that was distributed to nearly all sections of the world during World War II to provide an almost necessary item in both military and civilian rations at points where fresh yeast manufacture was not possible. Such commodities make an important contribution to improved food supplies during peace-times, as well, in the sense of enabling a nation to provide good food to all parts of the world, within the channels of normal commerce.

Yeast has made a major contribution to ridding the world of two major deficiency diseases, beriberi and pellagra. At levels of nutrition well above the zone where illness is imposed, yeast should be recognized permanently as a contributor to human health and economic food production.

C. G. KING,
The Nutrition Foundation, Inc.,
Chrysler Building, New York.

May, 1950.

Nitrogen Compounds of Yeast

H. E. CARTER
Division of Biochemistry, Noyes Laboratory of Chemistry
University of Illinois, Urbana

The major nitrogenous components of yeast are proteins and nucleic acids. The total nitrogen of dried yeast varies from 6 to 11 percent but the majority of the figures lie in the range 7 to 9 percent *(1-4)*. Purine nitrogen is usually 0.7 to 0.8 percent of the dry weight or about 10 percent of the total nitrogen *(1, 3, 5, 6, 7)*. Pyrimidine nitrogen composes about 4 percent of the total nitrogen *(8)*. On the basis of the "tetranucleotide" structure of ribose nucleic acid, the pyrimidine nitrogen should be half the purine nitrogen since the two purines (adenine and guanine) contain 10 nitrogen atoms while the two pyrimidines (cytosine and uracil) have only 5.

Yeasts contain also a variety of other constituents whose nitrogen content is only a small part of the total [glutathione, glucosamine *(8)*, lecithin and cephalin *(9)*, cytochrome *(10)*, vitamins, adenylic acid, adenosinetriphosphate, cozymase, etc.].

Yeast Proteins

Crude protein of yeast (N x 6.25) makes up 45 to 55 percent of the dry weight. However, this figure is obviously misleading in view of the high content of purine and pyrimidine nitrogen. von Soden and Dirr *(11)* concluded that only 80 percent of the total nitrogen was amino acid from studies of amino nitrogen in enzymatic hydrolysates of yeast. More recently Dirr and Decker *(3)* reported an even lower figure (64 to 76 percent) for "pure protein" of yeast. This apparent discrepancy is largely accounted for by the free amino acid fraction of yeast which has been reported to represent 7 percent of the total nitrogen *(12)*.

Attempts to isolate and characterize yeast proteins have met with little success. Yeast cells autolyze rapidly with consequent alteration in solubility and chemical nature of the proteins. Furthermore, after defatting and rupturing the cell membrane not all of the nitrogen can be extracted even with dilute alkali. Extraction with water removes a thermolabile and a thermostable protein. Subsequent extraction with a dilute salt solution removes a globulin and still further protein material is removed with alkali *(13, 14, 15)*. Csonka *(13)* considers all of these

fractions to be nucleoproteins. In view of the rapid autolysis of yeast cells it is not certain what relation these various fractions have to the proteins of the living yeast cell.

There are many reports in the literature on the amino acid composition of whole yeast and of various protein fractions. Many of these data, obtained with older and less reliable methods will be omitted (see reference 1). Table 1 summarizes certain of the more recent data on the amino acid composition of yeasts of various types.

TABLE 1

Amino Acid Composition of Yeasts

(Calculated to 16.0 percent of Nitrogen)

	A	B	C	D	E
Arginine	4.3	6.6	4.15	4.3	4.0
Cystine			1.0	1.3	
Glycine			4.54		4.2
Glutamic Acid			13.37		14.0
Histidine	4.8	3.6	1.71	2.8	2.0
Isoleucine	4.5	3.8	5.51	6.0	5.3
Leucine	6.8	5.9	7.57	7.3	7.0
Lysine	5.5	5.4	6.87	6.0	6.9
Methionine	1.2	0.9	0.83	2.0	1.3
Phenylalanine	3.8	3.1	3.86	4.1	3.8
Threonine	4.3	3.2	5.42	5.0	5.1
Tryptophane	1.1	0.8	1.65	1.8	1.5
Tyrosine			15.6	4.2	
Valine	5.0	4.5	6.08	5.3	5.9

A. S. cerevisiae [Stokes and Gunness *(16)*].
B. Rhodotorula rubra [Stokes and Gunness *(16)*].
C. Torula yeast [Courtesy of C. N. Frey].
D. Averages for several yeasts [Block and Bolling *(17)*].
E. Brewers' Type Yeast [Courtesy of C. N. Frey].

The results of Stokes and Gunness *(16)* were obtained by microbiological assay methods; those of Block and Bolling *(17)* by a combination of microbiological and chemical methods. The agreement among the various analyses is very good argument for the reliability of the procedures. Similar figures have also been reported by Edwards et al. *(18)*. The data in Table 1 show that yeast proteins are biologically complete containing all of the essential amino acids. The analyses of yeast are strikingly similar to those for casein and muscle protein (Table 2) except for the low level of methionine. The latter observation is in

accord with the improvement in biological value of yeasts known to result on the addition of methionine *(19)*.

TABLE 2 *(1)*

Percentage Composition of Yeast Protein, Muscle Protein and Casein

	Crude Protein of Yeast	Muscle Protein	Casein
Arginine	4.3	7.1	3.79
Histidine	2.8	2.2	1.81
Lysine	6.4	8.1	6.20
Tyrosine	4.2	3.1	6.01
Tryptophane	1.4	1.2	1.11
Phenylalanine	4.1	4.5	5.00
Cystine	1.3	1.1	0.26
Methionine	+	3.3	3.10
Threonine	5.0	5.2	4.40
Leucine	13.2 ± 2.6	12.1 ± 1.1	12.10
Isoleucine	3.4 ± 0.2	3.4 ± 0.2	....
Valine	4.4 ± 0.8	3.4 ± 0.4	7.00

Yeast Nucleic Acids

Yeast nucleic acid is readily prepared by extracting yeast with alkali at 0 to 10° *(20, 21)* or with 5 percent trichloroacetic acid at 90° *(22)*. On hydrolysis it yields adenine, guanine, cytosine, uracil, ribose, and phosphoric acid. Yeast nucleic acid had long been known as the classical example of a ribosenucleic acid [pentose nucleic acid (PNA), plasmonucleic acid *(23)*]. Recently Chargaff and Zamenjof *(24)* isolated a desoxyribosenucleic acid [desoxypentosenucleic acid (DNA), chromonucleic acid *(23)*] from yeast in very small amounts. Analytical data indicate that bakers' yeast contains 0.1 to 0.15 percent of desoxypentosenucleic acid *(24, 25)*. This is in marked contrast to the ribosenucleic acid content of 6.9 percent *(7)*.

For many years ribosenucleic acid was considered to be a tetranucleotide composed of one molecule each of adenylic, guanylic, cytidylic, and uridylic acids. However, strong evidence against such a formulation has accumulated in recent year. Loring and co-workers *(26)*, using a neurospora mutant to determine pyrimidines found that ribosenucleic acid contained a higher proportion of purine nucleotides than of pyrimidine. Furthermore, the molecular weights of various nucleic acid preparations range from 10,000 to 23,500 corresponding to 8 to 18 nucleotide units. It is possible that these materials represent degradation products of a much

higher molecular weight polynucleotide present originally in the yeast cells.

Further complications have been introduced by studies of the enzyme ribonucleinase. This enzyme hydrolyzes ribonucleic acid but only to a limited extent. All four mononucleotides have been isolated from the hydrolysis mixture *(27)*. However, the undigested portion contains a high purine but low pyrimidine content *(26)*. These data indicate that ordinary yeast ribosenucleic acid is not a "statistical" tetranucleotide but rather is a high-molecular weight probably inhomogenous *(26)* polynucleotide which does not involve a regular alternation of the four bases.

The presence of a high content of nucleic acid is a complicating factor in evaluating yeast as a source of protein. Not only the purine content but the phosphate also must be considered in planning nutritional experiments with yeast.

REFERENCES

1. Carter, H. E., and Phillips, G. E. *Federation Proc.* **3**, 123 (1944).
2. Dirr, K., and von Soden, O. *Biochem. Z.* **309**, 329 (1941).
3. Dirr, K., and Decker, P. *Biochem. Z.* **316**, 245 (1943-1944).
4. Braude, R. *J. Inst. Brewing* **39**, 206 (1942).
5. Dirr, K., and Decker, P. *Biochem. Z.* **316**, 239 (1943).
6. DeMan, T. J., and de Heus, J. G. *Voeding* **7**, 147 (1946).
7. von Euler, H., and Hahn, L. *Arkiv Kemi, Mineral. Geol.* **24**A No. 28 (1947).
8. Meisenheimer, J. *Z. physiol. Chemie.* **114**, 205 (1921).
9. Dirr, K., and von Soden, O. *Biochem. Z.* **312**, 263 (1942).
10. Borei, H., and Sjoden, A. *Arkiv Kemi, Mineral. Geol.* **16**A, No. 19 (1943).
11. von Soden, O., and Dirr, K. *Biochem. Z.* **312**, 252 (1942).
12. Roine, P. *Suomen Kemistilehti* **19**B, 37 (1946).
13. Csonka, F. A. *J. Biol. Chem.* **109**, 703 (1935).
14. Strain, H. H. *Enzymologia* **7**, 133 (1939).
15. Kazakov, K. *Khim. Referat. Zhur.* **1940**. No. 4, 40.
16. Stokes, J. L., and Gunness, M. *J. Bact.* **52**, 195 (1946).
17. Block, R. J., and Bolling, D. The Amino Acid Composition of Proteins and Foods, p. 304, C. C. Thomas, Springfield, 1945.
18. Edwards, L. E. et al. *J. Nutrition* **32**, 597 (1946).
19. Klose, A. A., and Fevold, H. L. *Proc. Soc. Exp. Biol. Med.* **56**, 98 (1944).
20. Johnson, T. B., and Harkins, H. H. *J. Am. Chem. Soc.* **51**, 1779 (1929).
21. U. S. Patent 2,387,040. *Chem. Ab.* **40**, 678 (1946).
22. Schneider, W. C. *J. Biol. Chem.* **161**, 293 (1945).
23. Pollister, A. W., and Mirsky, A. E. *Nature.* **152**, 692 (1943); **153**, 711 (1944).
24. Chargaff, E., and Zamenhof, S. *J. Biol. Chem.* **173**, 327 (1948).
25. Jenner, R., and Brachet, J. *Enzymologia* **11**, 222 (1943-1945).
26. Loring, H. S., Ordway, G. L., Roll, P. M., and Pierce, J. C. *Federation Proc.* **6**, 510 (1947).
27. Loring, H. S., and Carpenter, F. H. *J. Biol. Chem.* **150**, 381 (1943).

Enzymes in Yeast

M. J. JOHNSON
Department of Biochemistry, University of Wisconsin
Madison, Wisconsin

Enzymes, as such, are of no importance whatever in yeast used for feeding, since the enzymes must be inactivated by heat treatment in order to make the yeast suitable for food. The denatured yeast enzyme proteins present in heat-treated yeast, however, constitute a very important part of the food value of the yeast contributing amino acids and B-vitamins, the latter being present to a large extent as components of enzymes or coenzymes. Yeasts will often abstract vitamins from the growth medium in amounts greatly in excess of their possible requirements. Only in a few cases has systematic investigation been made to determine the extent to which such vitamins are present as enzymes or coenzymes. It appears certain, however, that in cases where the vitamin content is extremely high, that they are present in some loosely combined form.

Yeast contains probably 40 percent of true protein. Since the amount of purely structural proteinaceous tissue is probably small, the bulk of the yeast protein is undoubtedly enzyme protein. The figures in Table 1 are indicative of the amounts of some enzymes present in yeast.

TABLE 1

Concentration of Enzymes in Yeast

Enzyme	Yeast protein*	Reference
	%	
Carboxylase	2.5	*(1)*
Phosphoglyceraldehyde dehydrogenase	>0.62	*(2)*
Ethyl alcohol dehydrogenase	0.36	*(3)*
Hexokinase	0.32	*(4)*
Aminopolypeptidase	0.12	*(5)*

*Assuming 40% protein.

It would appear from the complexity and variety of the materials synthesized by yeast that hundreds of enzymes are to be found in the yeast cell. The enzymes of the respiratory systems probably comprise only a small percentage of the enzymes of yeast. Other enzymes found in yeast are those concerned with synthesis and breakdown of amino

acids and other nitrogen compounds, with the synthesis and hydrolysis of proteins and other compounds of high molecular weight, with the transport of metabolites across the cell membrane, and with the mechanism of synthesis of enzymes by means of which genetic structure is reflected in somatic constitution. Very little is known about these enzymes. The hydrolytic enzymes of yeast are components of these largely unknown yeast enzyme systems.

As far as is known, all the enzymes of yeast are intracellular enzymes which are liberated only on destruction of the cell. Living yeast ordinarily secretes no enzymes. Many yeast hydrolytic enzymes are known but they are all intracellular components used, in a known or unknown manner, in the internal cell economy.

Non-Respiratory Enzymes

The proteinase (or more likely, the proteinases) of yeast is, like the intracellular proteinase of most organisms, an enzyme of the so-called papainase group. It is activated by—SH compounds and other reducing agents, and inhibited by oxidizing agents. It attacks proteins best at or near their iso-electric point. Its function in the cell is unknown. It is perhaps the most important enzyme concerned in the autolysis of yeast. When yeast is subjected to conditions conducive to disorganization of the cell metabolic system, such as grinding, treatment with strong salt solutions, freezing and thawing, treatment with lipid solvents, or partial drying, the yeast cell is converted from a functioning organism to a chaotic mixture of enzymes and other compounds. The enzymes attack each other and all other available substrates, and the bulk of the cell substance is brought into solution. Since the largest single group of compounds in the cell is the proteins, the yeast proteinase is largely responsible for autolytic cell breakdown. The polypeptide fragments resulting from proteinase action are further broken down by the yeast peptidases. Organic phosphorus compounds are hydrolyzed by the numerous yeast phosphatases, and, in general, the organic compounds of the cell are hydrolyzed and otherwise degraded. Some enzymes, not attacked by the autolytic enzyme system, are best obtained from yeast autolyzates.

The number of hydrolytic enzymes found in yeast extracts is large, but none except perhaps the invertases, are present in sufficiently high concentrations to make yeast the preferred source of the enzyme.

Respiratory Enzymes

The best known respiratory system of yeast is the anaerobic system. The overall reaction carried out by this enzyme system is:

$$\text{Glucose} + 2\text{ADP} \longrightarrow 2C_2H_5OH + 2CO_2 + 2\text{ATP} + 2H_2O$$

The product of interest to the baker is carbon dioxide, to the distiller, ethyl alcohol, and to the yeast, adenosine triphosphate. The other compounds are merely necessary by-products in the process of ATP manufacture. The yeast, under anaerobic conditions, finds it necessary to convert sugar into ethyl alcohol and carbon dioxide to obtain the required energy to synthesize ATP from adenosine diphosphate and inorganic phosphate.

The mechanism by means of which the energy of conversion of sugar into alcohol and carbon dioxide is utilized for ATP synthesis is the Embden-Meyerhof-Parnas series of reactions, which are catalyzed by the anaerobic respiratory enzyme system of the yeast. The primary combination of inorganic phosphate takes place concomitantly with the oxidation step, and the ATP is formed by transphosphorylation during conversion of diphosphoglyceric acid into pyruvic acid.

The way in which phosphate bond energy is used in the great majority of the reactions involved in growth of yeast is unknown. The best expression of the present state of our knowledge is perhaps the following:

$$\text{Glucose} + \text{ATP} \longrightarrow \text{yeast cells} + CO_2 + H_2O + \text{ADP}$$

The yield of yeast cells obtainable under anaerobic conditions is approximately 5 grams of dry cells per 100 grams of fermented sugar. From this fact it may be calculated that the conversion of the carbon of one molecule of glucose into yeast carbon requires the expenditure of approximately 34 molecules of ATP.

If the yeast is grown in the presence of excess air, however, the cell yield is approximately 50 percent of the weight of the fermented sugar. Very evidently the yeast cell possesses some mechanism for making one molecule of glucose yield much more than two molecules of ATP. Aerobically the sugar utilized for energy is oxidized to carbon dioxide and water, rather than being simply converted to alcohol and carbon dioxide. Under highly aerobic conditions, the yeast brings into play its second respiratory enzyme system, the *aerobic respiratory system*. An approxi-

mate expression of the overall reaction carried out by the aerobic respiratory enzymes is the following:

$$C_6H_{12}O_6 + 30ADP + 6O_2 \longrightarrow 30ATP + 6CO_2 + 36H_2O$$

The assumption has been made that the oxidation of one molecule of glucose makes possible the synthesis of thirty molecules of ATP. It is doubtful that the actual number is exactly thirty. Aerobic phosphorylations have been studied much more intensively in animal tissues than in yeast, and the most recent evidence *(6)* indicates that in animal tissue, the number is approximately 30. At any rate, it is clear that the high cell yields associated with aerobic growth are a reflection of the greater number of phosphorylations accompanying the dissimilation of one molecule of glucose. When growing aerobically, the yeast actually uses sugar at a slower rate than when growing anaerobically, because of the much greater energy yield per molecule of sugar. The yeast cell, when oxygen is available, utilizes its aerobic enzyme system in preference to its anaerobic system.

The mechanism of the oxidation of glucose by yeast is not known, except in a very general way. There is good evidence for the existence of the tri-carboxylic acid cycle in yeast, and a functioning cytochrome system has long been known to exist. In neither animal tissue nor yeast is the mechanism of aerobic phosphorylation known.

The relative amounts of aerobic and anaerobic respiratory enzymes present in a yeast cell depend on both heredity and environment. If a given strain of yeast is cultivated aerobically, it will contain a higher ratio of aerobic to anaerobic enzymes than the same strain grown anaerobically. For example, *Torulopsis utilis* No. 3, grown in the author's laboratory under highly aerobic conditions, has a very active aerobic system but an extremely weak anaerobic system. Yet the same strain has been found to function very well in the anaerobic production of alcohol from wood sugar.* On the other hand, some yeasts are incapable of being adapted to anaerobic growth, while mutant strains of *S. cerevisiae* are known which are incapable of aerobic sugar oxidation, apparently lacking even a trace of an aerobic enzyme system.

In Table 2, the aerobic and anaerobic activities of two typical yeasts are given. In the case of the *Torula,* the resulting yeast had a very strong aerobic system and a weak anaerobic system. The commercial foil yeast, however, had a weak aerobic system even though grown aerobically.

---

*Work done by Dr. Harris and coworkers at Forest Products Laboratory.

TABLE 2

Respiratory Rates of Aerobically Grown Yeasts

	μl. gas/hour/mg. dry cells	
	T. utilis No. 3	Commercial foil yeast
Anaerobic CO_2 production	20	225
Aerobic oxygen uptake	212	60

The special case of bakers' yeast is of interest. The manufacturer of bakers' yeast must produce a yeast with a very active anaerobic enzyme system, so that it will produce carbon dioxide rapidly in doughs. But this yeast must be grown aerobically rather than anaerobically, since approximately ten times the yield is obtained. In order to prevent the aerobic growth conditions from yielding a product low in anaerobic activity and high in aerobic activity, a yeast strain is used which is genetically unable to attain a high ratio of aerobic to anaerobic enzymes. The strains used are, in fact, so anaerobic that even in the presence of excess oxygen alcohol is produced, since the deficient aerobic enzyme system is apparently incapable of producing ATP as rapidly as the cell requires it. Partially anaerobic growth, with resulting low yields is prevented by feeding sugar into the fermenter at a rate slow enough so that it can be handled by the aerobic system alone. Thus practically pure aerobic growth and high cell yields result.

The producer of yeast for food purposes can obtain higher yields with less trouble, since he need not select a strain with an inherently low aerobic system. The reason for the growing popularity of torula types as feed yeasts lies in part in the fact that these strains have, in general, a high capacity to develop a strong aerobic enzyme system. Good cell yields can be obtained without critical control of sugar concentration and aeration rate.

Because of the functioning of various B-vitamins in the respiratory enzyme systems of yeast, the question of the relative vitamin content of the aerobic and anaerobic enzyme systems naturally arises. The concentration of many vitamins in yeast is determined primarily by the vitamin content of the medium. The influence of enzymic constitution on vitamin content has never been specifically studied. It is interesting to note, however, that some correlation seems to exist between the amounts of niacin, riboflavin, and folic acid in the cells and the cell yield *(7)*. Since cell yield is a good indication of the extent to which the aerobic system is active, it might be concluded that the aerobic enzyme system requires

more nicotinic acid, but less riboflavin and folic acid, than does the anaerobic system. Such a generalization, however, cannot be made without further experimental work.

Summary

1. The enzymes of yeast resemble very much the corresponding enzymes from other sources. Yeast enzymes have no distinguishing features that set them apart from other enzymes.

2. Yeast protein is probably primarily enzyme protein.

3. Yeasts are provided with aerobic and anaerobic respiratory enzyme systems. The relative concentrations of these systems vary with the species and strain of yeast used, and with the manner in which the yeast is grown.

4. The vitamins in yeast are components of enzymes and coenzymes. There is an apparent correlation between the content of aerobic or anaerobic enzyme system and the amounts of certain vitamins present.

5. Many hydrolytic enzymes have been found in yeast, but the functions of most of them in the cell economy are obscure.

REFERENCES

1. Green, D. E., Herbert, D., and Subrahmanyan, V. *J. Biol. Chem.* **138**, 327 (1941).
2. Warburg, O., and Christian, W. *Biochem. Z.* **303**, 40 (1939).
3. Negelein, E., and Wulff, H. *Biochem. Z.* **293**, 351 (1937).
4. Kunitz, M., and McDonald, M. R. *J. Gen. Physiol.* **29**, 393 (1946).
5. Johnson, M. J. *J. Biol. Chem.* **137**, 575 (1941).
6. Cross, R. J., Taggart, J. V., Covo, G. A., and Green, D. E. *J Biol. Chem.* **177**, 655 (1949).
7. Peterson, W. H. *Vitamins and Minerals of Yeast. Yeasts in Feeding, Proceedings of the Symposium,* pp. 26-33. Garrard Press, Champaign, Illinois. 1950.

Carbohydrates in Yeast

CARL NEUBERG
Department of Chemistry, New York University
New York

The view that the yeast cell contains few carbohydrates *(1)* has been refuted by advancing investigations of the chemistry and biology of yeast. Soluble and insoluble carbohydrates have been found as permanent and transitory constituents of yeast. The best characterized of these are yeast cellulose, yeast glycogen and yeast gum. Other high molecular weight carbohydrates which occur are yeast dextrin and yeast mucilage. The carbohydrates are separated from one another by autoclaving yeast in water which results in solution of the glycogen and yeast gum, leaving the cellulose. Glycogen is extracted from yeast by diluted alkali, precipitated with alcohol and purified by repeated reprecipitation from water with alcohol. Admixed yeast gum can be precipitated with hot Fehling's solution *(2)*.

The cell membrane which constitutes 2.4 percent of the dry substance contains a polysaccharide which yields 34 percent D-glucosamine on hydrolysis with 10 percent acid *(3)*. In all likelihood this nitrogen-containing polysaccharidie is related to a *glycoprotein* rather than to chitin. It may be regarded as the precursor of the D-glucosamine hydrochloride which Meisenheimer *(4)* obtained in small amounts on boiling intact yeast cells with strong hydrochloric acid.

Of the carbohydrates occurring within the cell, *glycogen* represents the major portion. The yeast glycogen precipitated by alcohol is a white powder of specific rotation $[\alpha]\ D = +\ 192°$. It may be considered identical with animal glycogen. Molecular weight determinations give values of 2 to 4 million. A ramified structure and multiple branching has been postulated *(5)*.

Yeast glycogen is found to contain phosphorus. Dialysis, electrodialysis and ultrafiltration may reduce the P_2O_5 content but will not remove it completely. This P is bound in an ester-type linkage. It has not been established whether or not some remnant of hexose phosphate not dephosphorylated during its condensation to the polysaccharide may be involved. According to Samec *(6)* the P_2O_5 content of highly purified natural glycogen amounts to 0.72 percent. This refers to the sol fraction of the glycogen which may be separated according to Karrer *(7)*. More recently Jeanloz *(8)* reports 0.2 percent P_2O_5.

Essentially glycogen belongs to the soluble constituents of the yeast cell, although part of it is loosely bound to other substances, especially proteins, either by adsorption or coordination. The free and bound forms of glycogen are easily hydrolyzed by both acids and enzymes.

Glycogen is a reserve material for yeast. The quantity present undergoes considerable fluctuations since glycogen is subject to rapid mobilization. Glycogen can be regarded as the main source of the alcohol and carbon dioxide produced by the so-called autofermentation of yeast. This postulate is supported by the fact that yeast completely freed from glycogen can no longer undergo autofermentation *(9)*. It is estimated from the amount of CO_2 produced during autofermentation that up to 30 percent of glycogen may be contained in the yeast cell *(10)*.

Added glycogen is not fermented by intact yeast since it does not diffuse through the intact cell membrane to the interior where fermentation takes place. As soon as this barrier is pierced, however, contact is established between zymase and glycogen and the glycogen is fermented in the normal way (presumably under participation of glycogenases).

The *insoluble polysaccharide* from yeast differs from glycogen in all important properties. It forms the main constituent of the yeast cellulose *(11)*.

This polysaccharide also requires very concentrated HCl for its hydrolysis. It differs, however, from both true cellulose and glycogen in that it contains 1, 3 linkages. The polysaccharide has been isolated from *Saccharomyces cerevisiae (12)*. It is obtained in a yield of 7.9 percent on a dry weight basis and is nonreducing and insoluble. Upon hydrolysis, it yields glucose in the form of β-glucose. This polysaccharide belongs to the closed chain type and has a molecular weight of approximately 6500. Another polysaccharide extracted from yeast by treatment with diluted alkali *(13)* appears to be related. It is said to be a 1, 3-glucan with a β-configuration.

Yeast gum has played an essential part in the chemistry of yeast and of its enzymes *(14, 15)*. Like glycogen yeast gum appears to be a reserve carbohydrate of yeast, but it differs from it in all other respects. It forms a hygroscopic powder with an optical rotation $[\alpha]\ D = +\ 89°$, and is not attacked by any amylase nor by takadiastase or emulsin. Rather strikingly, however, it is said to be fermented by juices or acetone-dried preparations of bottom yeast, while on the other hand, it accumulates during autofermentation of yeast *(16)*. No gum is contained in yeast press juice nor in rapidly prepared yeast autolyzates. Yeast gum has

been considered to be a mannan *(17)* and a gluco-mannan *(18)*. If sufficiently purified, the gum is solely a mannan. The mannan consists entirely of α-mannopyranose residues, joined by α-glucosidic linkages *(19)*.

The existence of *yeast-levulan* is doubtful *(20)* and the same is true for the methylpentosan mentioned by Oshima *(20)*. The *dextrin-like material*, sometimes found in natural wines, might be considered to be derived from yeast polysaccharides *(20)*.

An interesting relationship with other mucilaginous materials is shown by observations on a symbiosis of *Saccharomyces cerevisiae* with *Leuconostoc mesenteroides (21)*. In mixed cultures the maximum yield of *dextran* was obtained in 40 hours, whereas the pure culture of *Leuconostoc* required 10 days for maximum production. Probably the dextran formation is stimulated by a factor produced by living yeast cells.

Besides glycogen true *amylose* also may occur in yeasts. This has been demonstrated, at any rate, in the case of *Torulopsis*. This material was found to contain at least two fractions, one being a pentosan, the other an amylose. The amylose could be formed only at a pH of 5 or lower whereas production of the pentosan may take place in the alkaline range *(22)*. The nature of the pentosan just mentioned has not been elucidated as yet. Its investigation would be of interest since no other pentose polysaccharides have ever been observed in yeast.

The occurrence of the pentose, *d-ribose*, in a mixed glucosidic and ester-type linkage is well known. The pentose is a constituent of yeast nucleic acid and is present in considerable amounts. Apparently there also occur small quantities of *desoxyribose* in conformity with a small thymonucleic acid content *(23)*.

The alcohol corresponding to ribose, *ribitol* or *adonitol*, is a constituent of riboflavin and the coenzymes. *Dulcitol* is another sugar alcohol, which like ribitol is optically inactive due to its mesostructure. The presence of dulcitol in yeast grown in wood-sugar solution has been reported *(24)*. Perhaps the dulcitol is formed by phytochemical reduction of the α-galactose of the culture medium *(25)*.

Other carbohydrates such as the monophosphate of ketoheptose *(26)* and thiomethylpentose *(27, 28)* have been reported to occur in yeast but their functions are still unknown.

The carbohydrate trehalose has been observed to accumulate in bakers' yeast *(29)*. Although previously reported to be formed solely by top yeast *(30)*, this nonreducing sugar has been obtained with brewers'

bottom yeast *(31)*. It is not decided whether the trehalose monophosphate present among the phosphorylated products of sugar fermentation is a precursor or derived from the free disaccharide *(32)*. In certain yeasts trehalose may accumulate up to 10 percent or more of the dry weight. This would imply quantities almost as big as those of the reserve carbohydrate glycogen. Unlike this reserve material, however, trehalose will not undergo autofermentation. Added trehalose is fermented by yeast maceration juice and many living yeasts, under certain conditions *(33)*, even more readily than glucose. Although trehalose is an α-glucoside, it will not be cleaved under any circumstances by typical α-glucosidases as e.g., maltase; a specific enzyme present in yeasts, the trehalase, is required for its hydrolysis.

Hexose phosphates are present also as constituents of the interior of the yeast cells *(34, 35)*. It was postulated that the hexose phosphates are formed by an intracellular process in the living cell *(36)*, but it is possible also with living yeast *(37)*.

Some insight into the question of the purpose of the considerable storage of diverse carbohydrates in yeast has been provided by the investigations of Lindegren *(38)* and Musfeld *(39)*. Glycogen-free and dormant yeast cells take up about seven times as much glucose as the same quantity of yeast would utilize for fermentation and respiration under otherwise identical conditions. The greater part of the sugar is transformed into glycogen. In the induction of dormancy in yeast cells and the conditions of reactivation, carbohydrate storage is essential.

Summary

Until recently little attention has been paid to the occurrence, properties and behavior of carbohydrates present in yeast cells. The advances made in yeast research have changed this situation. An outline is given of the present state of our knowledge of the properties and biochemical transformations of carbohydrates inherent in yeast cells.

REFERENCES

1. v. Nägeli, C. *Sitzber, kgl. Bayr, Acad. Wissensch., Mathem-Physik. Klasse.* **8**, 166 (1878).
2. Ling, A. R., Nanji, D. R., and Paton, F. J. *Inst. of Brewing* **31**, 316 (1925); *Chem. Centralbl.* II, 2170 (1925).
3. Schumacher, J. *Centralbl. f. Bacteriol.* I, 108, 193 (1928); *Chem. Centralbl.* I, 547 (1929).
4. Meisenheimer, J. *Z. physiol. Chemie* **104**, 234 (1919).
5. Meyer, K. H. *Advances in Enzymol.* **3**, 109 (1943).
6. Samec, M., and Isajevic, V. *C. r.* **176**, 1419 (1923).

7. Karrer, P. *Einfuehrung in die Chemie der polymeren Kohlenhydrate*, Leipzig, Akademische Verlagsgesellschaft. p. 92 (1925).
8. Jeanloz, R. *Helv. Chim. Act.* **27**, 1501 (1944).
9. Buchner, E., and Mitscherlich, S. *Z. physiol. Chemie* **42**, 554 (1904).
10. Quoted according to v. Lippmann, E. O. *Chemie d. Zuckerarten*, Braunschweig, p. 580 (1904).
11. Salkowski, E. *Ber. chem. Ges.* **27**, 497, 925 (1894).
12. Hassid, W. Z., Joslyn, M. A., and McCready, R. M. *J. Am. Chem. Soc.* **63**, 295 (1941).
13. Barry, V. C., and Dillon, T. *Proc. Roy, Irish Acad.* **49**B, 177 (1943) ; *Chem. Ab.* **38**, 956 (1944).
14. Salkowski, E. *Ber. chem. Ges.* **27**, 3325 (1894) ; *Z. physiol. Chemie* **69**, 466 (1910).
15. Errera, L. *Bibliographie de glycogène et de paraglycogène*, Bruxelles (1905). See also v. Nägeli, C. *J. pract. Chemie* (N. F.), **125**, 403 (1878).
16. Warkany, J. *Biochem. Z.* **150**, 271 (1924). See also Gottschalk, A. *Z. physiol. Chemie* **153**, 215 (1926) ; Kraut, H. et al., *Ber chem. Ges.* **60**, 1644 (1927).
17. Hessenland, F. *Z. f. Rübenzuckerindustrie* **42**, 671 (1892) ; *Chem. Centralbl.* II, 572 (1892).
18. v. Euler, H., and Fodor, A. *Z. physiol. Chemie* **72**, 339 (1911).
19. Haworth, W. N., Heath, R. L., and Peat, S. *J. Chem. Soc.* 833 (1941).
20. Literature quoted in v. Lippmann, E. O. *Die Chemie der Zuckerarten*, Braunschweig, 160, 216, 643, 807 (1904). Abderhalden, E. *Biochem. Handlexicon.* II, 35-36 (1911).
21. Stacey, M. *Nature* **149**, 639 (1942).
22. Aschner, M., Mager, J., and Leibowitz, J. *Nature* **156**, 295 (1945).
23. Chargaff, E., and Zamenhof, S. *J. Biol. Chem.* **173**, 327 (1948).
24. Fink, H., and Just, F. *Biochem. Z.* **296**, 307 (1938) ; *Chem. Ab.* **33**, 677 (1939).
25. Neuberg, C. *Advances in Carbohydrate Chem.* **4**, 75 (1949).
26. Robison, R., Macfarlane, M. G., and Tazelaar, A. *Nature* **142**, 114 (1938).
27. Suzuki, U., Odaka, S., and Mori, T. *Biochem. Z.* **154**, 278 (1924) ; **162**, 413 (1925).
28. Falconer, R., and Gulland, J. M. *J. Chem. Soc.* 1912 (1937).
29. Koch, E. M., and Koch, F. C. *Science* **61**, 570 (1925).
30. Tanret, G. *Bull. Soc. Chim. Biol.* **13**, 598 (1931) ; *Chem. Centralbl.* II, 1712 (1931). See also Steiner, A., and Cori, C. F. *Science* **82**, 422 (1935).
31. Veibel, S. *Biochem. Z* **239**, 350 (1931) ; **252**, 305 (1932). See also Kluyver, A. J., and van Roosmalen, L. M. ibid. **245**, 13 (1932) ; Brandt, K. M. ibid. **309**, 190 (1941).
32. Hestrin, S. Wallerstein Labs. Commun. **11**, 204 (1948).
33. Myrback, K., and Vasseur, E. *Z. physiol. Chemie* **277**, 171 (1943).
34. Macfarlane, M. G. *Biochem. J.* **33**, 565 (1939).
35. Suomalainen, H,. and Arhimo, E. *Science* **105**, 17 (1947).
36. Harden, A. *Alcoholic Fermentation*, 4th edition, London (1932).
37. Neuberg, C. *Biochem. Z.* 280, 163 (1935).
38. Lindegren, C. C. *Arch. Biochem.* **8**, 119 (1945).
39. Musfeld, W. *Ber. schweiz. bot. Ges.* **52**, 583 (1942).

Yeast Lipids and Their Biological Synthesis

J. C. HOOGERHEIDE
Pabst Brewing Co., Milwaukee, Wisconsin

The wide distribution of lipids in biological matter seems to indicate that synthesis as well as utilization of fat by a living organism are subjected to biological principles of a general nature which are valid for microorganisms as well as for the higher plants or animals. A fundamental study of fat synthesis by yeasts, a study which can be made under much better controlled conditions than is possible with animals, might therefore contribute greatly to our understanding of the mechanism and chemistry of fat synthesis and fat metabolism in general.

Unfortunately biological fat synthesis and metabolism, even when studied on microorganisms and under controlled conditions, is a very difficult problem and our knowledge accumulated over more than a hundred years of study of this phenomenon is still very limited.

Composition of Yeast Lipids

Lipids from several yeast and mold species have been isolated, investigated and analyzed in great detail. It has been found that yeast lipids, just like those of other living organisms, can be classified into three main groups namely, *phospholipids, sterols and glycerides of a number of fatty acids.*

Phospholipids are present in yeasts only in small quantities (at most 1 or 2 percent of the dry weight) and can be separated from the rest of the lipid fraction by means of their acetone insolubility. Lecithin and cephalin comprise 75 and 25 percent of the total yeast phospholipids respectively *(1)*.

The mechanism or even the sequence of the synthetic processes involved in phospholipid synthesis are still obscure. It is possible that phospholipids play a part in the synthesis or metabolism of fat, but this is by no means certain.

Sterols occur in yeasts esterified with fatty acids. They compose the unsaponifiable fraction of yeast lipids, and may comprise up to 50 percent of such lipids. Ergosterol is by far the most important sterol present in

yeast. Other sterols, such as zymosterol and cerevisterol, are found in small quantities.

The physiological function of sterols in yeasts is still unknown. Minimum amounts of both sterols and phospholipids are apparently essential as parts of the structure of the yeast cell.

Many microorganisms are able to accumulate, over and above the small amounts of sterols and phospholipids which they contain, considerable amounts of fats in the form of triglycerides. This fat functions as a reserve material. The amount of reserve fat in most yeasts is usually small but in certain yeast species it may be as high as 60 percent of the dry weight of the yeast.

Table 1 summarizes some analytical data obtained during the past 15 years on the fat isolated from several yeast species.

The conclusion that may be drawn from the extensive work on analyses of yeast fat is that, in general, its composition does not differ greatly from that of the vegetable oils. It contains oleic acid as the main

TABLE 1

Analysis of Yeast—Fat

Analysis	Saccharomyces cerevisiae (2)	(3)	Blastom. dermatitides (4)	Torula utilis (5)	Fat-yeast No. 72 (6)
	%	%	%	%	%
Fat content	7		10	6	30
Saponification No.	110	157	192	181	206
Acid No.	29	108	45	102	68
Iodine Value	61	130	106	121	63
Unsaponifiable mt.	47	20	8	12	2
Fatty acids	47	66	88	77	
Myristic (C14)				0.3	0.1
Palmitic (C16)	13	14	11	8	26
Stearic (C18)	4	8	6	4	6
Saturated C20, C22				0.2	5
Hexadecenoic (= C16)	19			8	1
Oleic (= C18)	58	67	63	22	55
Linoleic (= C18)		4	21	50	6
Linolenic (= C18)				4	0.7
Unidentified	9	7		5	1

component, and lesser amounts of palmitic, stearic and linoleic acids. Highly unsaturated fatty acids such as linolenic acid are usually absent or present in small amounts only.

Often a large proportion of free fatty acids are present, evident as a high "acid number." The "iodine number" of crude yeast lipids is usually quite high. This is not due to a large fraction of unsaturated fatty acids but to the presence of a compound called squalene which is a highly unsaturated terpenoid hydrocarbon ($C_{30}H_{50}$).

The large discrepancies in analyses of yeast lipids by different investigators are usually attributed to the marked differences in the technic of isolating the lipid fraction from the yeast or to the technic of analysis. A much more logical explanation, however, derives from the fact that the composition of yeast fat of the same strain was found to vary greatly with such factors as composition of the culture medium, the carbohydrate concentration, the age of the culture, the pH of the medium and the incubation temperature. The influence of these factors on the lipid composition has never been studied systematically. Such a study, especially with high fat yeasts, is highly desirable since progress in elucidating the mechanism and chemistry of biological fat synthesis depends upon it.

Lipid Formation By Yeasts

Conditions under which yeasts are normally cultivated result in low fat contents. Under the proper conditions, however, all yeasts and molds are able to synthesize and to accumulate large amounts of fat, varying from 10 to as much as 60 percent of their own weight on a dry basis. For instance, bakers' yeast, suspended in a sugar solution and thoroughly aerated at a temperature of 25° to 30° C. and a pH of 4 to 6, may easily increase its fat content from a normal of 5 to 6 percent to 15 to 20 percent fat.

The process of lipid formation by yeasts is most active after growth has practically ceased. Lipid formation is an aerobic process, requiring an abundant supply of oxygen and inorganic phosphate, a high concentration of carbohydrates or other assimilable respiratory substrates, and a low concentration of assimilable nitrogen.

Although in general all yeasts and molds are able to synthesize fat from carbohydrates under the conditions mentioned, most of them, including bakers' and brewers' yeasts have a low conversion factor. This is due in part to the large portion of carbohydrate required as an energy source and as building blocks to produce the yeast crop for subsequent fat synthesis. Moreover most yeast species synthesize, in addition to fat,

other reserve material such as glycogen from carbohydrate. Finally, part of the energy from carbohydrate oxidation often goes to waste in the form of heat produced.

The highest conversion factor (fat-coefficient) of carbohydrate into fat can be expected with yeast strains which are able to accumulate large amounts of fat per cell and at the same time synthesize a minimum of other reserve materials from the carbohydrate available. Actually it was found that even with the best fat producing yeast strains known at present, the total fat yield obtained in growing yeast cultures does not exceed 15 to 18 grams of fat per 100 grams of sugar consumed.

Since yeast fat represents about two and one-half times as much potential energy as an equivalent amount of glycogen, an accumulation of fat by yeasts means building up a potent energy reservoir, far superior for the yeast than the usual glycogen storage. However, from a production standpoint this means that the yeast yield when cultivating high fat yeasts can always be expected to be considerably below that obtained by cultivation of high protein yeast from the same amount of carbohydrate.

In general, it can be said that an increase in the fat content of the yeast cell is accompanied by a marked decrease in protein content, far in excess of the relative decrease due to increased weight. Whereas the normal protein content of yeast is of the order of 50 percent of its dry weight, it was found that *Rhodotorula gracilis* with 60 percent fat, contains only 14 percent protein. Similarly *Endomyces vernalis* with 45 percent fat and Starkey's fat-yeast with 60 percent fat contain in each instance only 9 percent protein. Both of these yeast species, however, when cultivated under conditions where no fat is formed, have a normal protein content of over 40 percent. The carbohydrate content of high fat yeast is of the same order as that of normal yeasts and varies little with the fat content of the cell.

The high concentration of fat present in high fat yeasts cannot be extracted from the cell by the usual procedures with fat solvents. Apparently, the fat is tied up with the protein and/or carbohydrate of the protoplasm. This linkage must be broken by boiling with dilute acids, by autolysis or by proteolysis before the fat can be liberated and extracted with solvents.

Feeding experiments with *Endomyces vernalis* yeast *(7)* showed that dogs utilized only 58 percent of the total fat present in the yeast, whereas a pre-digestion of the yeast cells increased the fat digestibility for the dog to the normal rate of 90 percent. This indicates that the animal body

encounters some difficulty in breaking this linkage. Once this has been accomplished yeast fat seems to be as digestible as plant or animal fats.

During the past thirty years a considerable amount of research has been devoted to the development of a practical process for microbiological fat synthesis. The ideal organism for microbiological fat synthesis, generally speaking, would be one that:

(a) reproducibly accumulates large amounts of fat, e.g., 50 to 60 percent of its dry weight, has a high conversion factor (preferably over 18 percent), and at the same time gives a high yeast yield;

(b) grows on agricultural or industrial waste products and can be cultivated submerged in tanks similar to bakers' yeast;

(c) grows fast, is not susceptible to contamination and can be recovered easily from the culture medium, preferably by sedimentation or simple filtration;

(d) contains fat which is readily released and digestible and has an acceptable taste.

As might be expected, such an ideal fat-producing organism is not known at present.

Two good fat-producing species, *Endomyces vernalis* (28 percent fat content) and *Oidium lactis* (50 percent fat content) could not be cultivated under submerged conditions. A number of other high fat yeast strains however were found to be suitable for submerged cultivation. One of these is an unidentified strain, isolated from soil *(8)*. This yeast may contain up to 62 percent of fat and has a conversion factor of 12 to 13 percent. Another strain is *Torulopsis lipofera,* originally isolated from soil *(9, 10)*. This yeast when cultivated submerged may contain up to 43 percent fat. A third yeast strain is *Rhodotorula gracilis (11)*. This yeast, grown in aerated tanks on pilot plant scale, contained 50 to 60 percent fat. A fourth yeast is *Candida reukaufii,* isolated from the leg of an ant and yielding 10 to 15 grams of fat per 100 grams of carbohydrate when grown submerged *(12)*. Other fat producing strains of lesser importance include: *Torulopsis pulcherrima* and *Rhodotorula glutinis (13)*.

True molds have also been studied extensively for their fat-producing properties. To mention only a few, the following species are able to accumulate large amounts of fat: *Penicillium javanicum (14, 15), Fusarium lini (16),* and *Aspergillus fischeri (17)*.

Damm *(16)* reveals that a strain of *Fusarium* can be cultivated on industrial waste products, submerged in tanks yielding a mycelium that

contained 50 percent fat, with a conversion factor of 12 to 15 percent. Bernhauer *(18)* also reports that an unidentified *Mucor* strain, cultivated submerged, yielded a mycelium with 64 percent fat, with a conversion factor of 13.6 percent.

Several investigators have stressed the point that biological fat synthesis with yeasts under normal economical conditions is unfeasible. However, others have pointed out that most of the research was done on surface cultures of yeasts or molds and that a reexamination of many fat-producing strains under submerged conditions is advisable. It is generally agreed that even under submerged conditions the carbohydrate source should be an agricultural or industrial waste product to make the process economically possible.

Summary

1. Lipids are present in yeasts as phospholipids, as esters of sterols and in the form of triglycerides.

2. Although, generally speaking, all yeasts and molds can synthesize fat from carbohydrates, a number of special strains have been found which are able to accumulate as much as 60 percent of their dry weight as fat, and have conversion factors of carbohydrate to fat of up to 18 percent.

3. The composition of high-fat yeast fat resembles very much that of the plant fats and has all the properties of a good quality edible fat, when liberated from the cell.

REFERENCES

1. Salisbury, L. F., and Anderson, R. J. *J. Biol. Chem.* **112**, 541 (1936).
2. Newman, M. S., and Anderson, R. J. *J. Biol. Chem.* **102**, 219 (1933).
3. Taufel, K. et al. Lebensm. *Untersuch. u. Forsch.* **72**, 394 (1936).
4. Peck, R. L., and Hauser, C. R. *J. Am. Chem. Soc.* **60**, 2599 (1938).
5. Reichert, R. *Helv. Chim. Acta* **28**, 484 (1945).
6. Hilditch, T. P. *Adv. Enzymol.* **8**, 320 (1948).
7. Völz, W. et al. *Biochem. Z.* **114**, 111 (1921).
8. Starkey, R. L. *J. Bact.* **51**, 33 (1946).
9. den Dooren de Jongh, L. E. *Ned. Tydschr. Hyg.* **1**, 136 (1926).
10. Kleinzeller, A. *Biochem. J.* **38**, 480 (1944).
11. Enebo, L. et al. *Arch. Biochem.* **11**, 383 (1946).
12. Rippel, A. *Naturwiss.* **31**, 248 (1943).
13. Nilsson, R. et al. *Svensk. Kem. Tid.* **55**, 41 (1943).
14. Ward, G. E. et al. *Ind. Eng. Chem.* **27**, 318 (1935).
15. Lockwood, L. B. et al. *Z. Bakt. Parasitenk.* **90**, 411 (1934).
16. Damm, H. *Chem. Ztg.* **67**, 47 (1943).
17. Prill, E. A. et al. *Biochem. J.* **29**, 21 (1935).
18. Bernhauer, K. *Eng. der Enzymf.* **9**, 297 (1943).

Vitamins and Minerals of Yeast

W. H. PETERSON
Department of Biochemistry, University of Wisconsin
Madison, Wisconsin

Yeast is one of the richest sources of the B-vitamins in our food supply. It also contains large amounts of ergosterol, which on irradiation becomes a rich source of vitamin D.

Not only is it valuable as a food because of its high nutritive content but also because of the high efficiency with which yeast converts crude materials into an edible product. Yields of dry yeast equal to about 50 percent of the sugar contained in molasses, sulfite-waste liquor, wood sugar, and waste-fruit products can be obtained regularly. Yeast has therefore received much consideration in the planning of food programs, especially in those countries where there is a surplus of low-grade sugar materials.

Vitamin Content

Striking variations are found in the vitamin content of yeasts. Although inherent and uncontrollable factors are partially responsible, the greatest variations are caused by the differences in environmental conditions under which yeasts are grown. Indeed, within limits, conditions of growth may be so altered as to produce a yeast with a designated vitamin content. The yeast, *Saccharomyces cerevisiae* (bakers', brewers', primary grown), is remarkably responsive to additions of thiamine, niacin and biotin to the medium and, conveniently for the producer, incorporates these vitamins into the yeast cell.

In Table 1 are collected data on the B vitamins of various types of yeast produced in the United States on an industrial scale and two others that have been produced commercially in Germany.

The figures for the *S. cerevisiae* types of yeast (bakers', brewers', and primary grown) show the greatest variation for thiamine and niacin and the least for riboflavin and pantothenic acid.

Thiamine Content

Brewers' yeast is conspicuously higher in thiamine content than bakers' yeast as normally produced. However, an enriched bakers' yeast containing 600 mcg. of thiamine per gram of dry yeast has been produced commercially. Foil or household yeast appears to be higher in

TABLE 1

*Vitamin Content of Yeasts (mcg./g. dry matter)**

Yeast	Thiamine	Ribo-flavin	Niacin	Panto-thenic acid	Vitamin B_6 (pyri-doxine)	PABA	Folic acid	Biotin	References
I. Commercial Samples									
Bakers', compressed	11-60	25-80	293-482	150-280	25-39	24-167	23-54	0.8 -2.4	1-12
Bakers', foil	88-139	57-59	256-336	131-157	.....	28-39	.....	0.56-1.47	8, 9
Bakers', active dry	7-29	31-70	180-351	100-150	.....	35-67	.....	0.43-1.52	3, 9, 13
Brewers', undebittered	110	38	400	80	31	.....	30	1.1	14
Brewers', debittered	104-250	25-80	300-627	72-86	23-40	15-40	19-30	1.1	1, 4-7, 11, 12, 14-18
Primary Grown	120-205	40-80	250-700	112-150	32-40	10-16	.....	2.5	1, 3, 5
T. utilis, grown on molasses	22	54	440-490	.....	.....	.....	.....	.....	12
T. utilis, grown on sulfite waste	5-14	33-70	153-467	40-123	.....	.....	.....	2.5	19
II. Experimental yeasts, grown aerobically									
S. cerevisiae	28-41	39-62	277-568	.....	.....	11-62	19-36	0.45-3.6	20-22
T. utilis	6-42	26-68	213-690	106-180	28-47	15-40	7-15	1.1 -3.6	16, 20-24
C. arborea	13-33	46-70	301-580	.....	.....	11-21	12-26	0.24-3.2	20-23
O. lactis	12-29	40-55	186-248	.....	.....	.....	6-15	1.3 -2.1	20-23

* The data in this Table represent, presumably, unfortified yeasts, *i.e.*, yeasts to which the vitamin has not been added or yeasts that have not been grown with the object of producing a yeast of high vitamin content. However, one cannot be sure that the sample is an unfortied product, as authors do not always give this information.

thiamine than bakers' trade yeast. Active dry yeast, on the other hand, seems to be considerably lower in thiamine than the other two types. The other yeasts, *Torula utilis, Candida arborea* and *Oidium lactis,* have substantially the same thiamine content as unfortified bakers' yeast.

The effect of adding thiamine to the medium, on the uptake of thiamine by bakers' yeast, is shown in Table 2. Inspection of the last

TABLE 2

Uptake of Thiamine by S. cerevisiae (25)

Yeast	Yield dry basis	Thiamine		
		Added	In yeast, dry	Absorbed
	g./l.	mg./l	mcg./g.	%
Bakers', grown in laboratory	7.4	None	55	....
Bakers', grown in laboratory	7.2	5	610	80
Bakers', grown in laboratory	7.1	25	2965	84
Bakers', grown in laboratory	6.2	313	6260	8
Commercial bakers'	8.3	None	19	....
aerated 12 hrs. in spent medium	7.9	5	600	92
	8.3	25	2428	80

Medium, g./l.: beet molasses 50, corn steep liquor 9, diastatic malt extract 21, $(NH_4)_2HPO_4$ 1. Medium was well-aerated during fermentation.

column shows that supplementation up to 25 mg. per liter resulted in recoveries of 80 percent or more of the added thiamine in the laboratory-grown yeast. Practically the same response to added thiamine was obtained from commercial bakers' yeast by aerating it in a spent medium to which thiamine was added. No greater weight of cells was present at the end of this time but more than 80 percent of the added thiamine had been taken up.

Riboflavin

The riboflavin figures in Table 1 vary somewhat less than those for thiamine, probably because yeasts, especially the Saccharomyces type, are not much affected by the riboflavin content of the medium. Riboflavin is not an essential growth factor for these yeasts, as they synthesize this vitamin.

Niacin

Table 1 shows that niacin is the most abundant vitamin in yeast. It is present in amounts of from 5 to 20 times that of thiamine and 2 to 5 times that of pantothenic acid. As in the case of thiamine, brewers' yeast contains somewhat more niacin than bakers' yeast, but the difference is less marked than for thiamine. Active dry yeast seems to be lower in

niacin, as well as thiamine, in comparison to bakers' press-yeast. *T. utilis* and *C. arborea* contained niacin in approximately the same range as the Saccharomyces yeasts, but *O. lactis* was conspicuously low in niacin.

Added niacin, like thiamine, is absorbed by many yeasts. The uptake of niacin by *S. cerevisiae* (Table 3) indicates that the niacin content may

TABLE 3

Effect of Added Pantothenic Acid and Niacin on Uptake by S. cerevisiae (9)

Addition	Yeast, mcg./g. dry matter	
	Pantothenic acid	Niacin
mg. l.		
None	154	502
1, pantothenic acid	157	473
10, pantothenic acid	161	512
6, niacin	138	1125
60, niacin	188	2930

Medium, gm./l.: cane molasses, 50; diastatic malt extract, 21 gm.; corn steep liquor, 9; $(NH_4)_2HPO_4$, 1. Medium was well aerated during fermentation.

be more than doubled when niacin is added to the medium at a 6 mg. per liter level and increased nearly sixfold at the 60 mg. per liter level.

Van Lanen *(26)* reports that the niacin content of bakers' yeast may be increased two- to threefold by adding niacin (10 mg./liter) to the medium. Brewers' yeast, when grown aerobically, took up very little added niacin. *T. utilis* approximately doubled its niacin content when the niacin content of the medium was increased threefold. *T. lactosa* and *C. guilliermondi* took up little or no niacin.

Pantothenic Acid

With the exception of niacin, pantothenic acid is present in yeast in larger quantities than any other known B vitamin. Table 3 shows that no significant change in the pantothenic acid content of bakers' yeast was obtained by adding pantothenic acid to the medium. This result is rather unexpected since pantothenic acid is a growth factor for this yeast and it would seem logical to expect the compound to be taken up readily by the cells.

Vitamin B_6 (pyridoxal, pyridoxamine, pyridoxine)

Data on the B_6 content of industrial yeasts, *e.g.*, active dry-yeast and *T. utilis*, are meager or conspicuously absent. Most yeasts do not require B_6 but some species show a response to added B_6. *S. carlsbergensis*, a yeast that requires B_6 for growth, was reported to contain only 1.9 mcg. (as pyridoxal) per gram of dry cells when supplied with insuffi-

cient B_6 for optimum growth (1 mcg./liter of medium) and 14.5 mcg. (as pyridoxal) per gram when 1 mg. of pyridoxal was placed in the medium—a more than sevenfold increase *(27)*.

p-Aminobenzoic Acid

Yeast synthesizes large quantities of PABA, 70 to 90 percent of which is found in the medium. Synthesis of PABA by *S. cerevisiae* ranging from 150 to 600 mcg. per gram of dry cells has been reported *(8)*. *Mycotorula lipolytica* and some other species have been found to synthesize over 1,000 mcg. PABA per gram of dry cells *(22)*. *T. utilis* on the other hand produces very little PABA (40 mcg./g).

The variation in PABA content of the yeasts listed in Table 1 is probably concerned with the synthesis and secretion of this nutrient. The amount of PABA remaining in the cell may well be determined by the age of the cell and external conditions in the medium.

Folic Acid (pteroylglutamic acid and related compounds)

The data for this vitamin are meager, and there are no figures for some of the commercial products. The values given are low and in the same range as for B_6. No data have been found regarding the effect of increasing the folic acid content of the medium on the content of this vitamin in the yeast.

Biotin

Though widely distributed in nature, biotin is found only in small quantities in yeast (1-2 mcg./g.—1/500 as much as the most abundant vitamin, niacin). The figures vary considerably, probably, because of the varying biotin content of the medium in which the yeasts were grown. Yeasts take up biotin as readily as they absorb thiamine.

Table 4 gives data showing the response of three food yeasts to added biotin. With larger and larger additions of biotin to the medium, the

TABLE 4

Uptake of Biotin by Three Yeasts (23)

Biotin added mcg./l.	Biotin mcg./g. dry cells		
	S. cerevisiae 53	*T. utilis*	*C. arborea*
0	0.54	2.8	0.36
80	11.8	4.4	8.6
640	116.0	4.8	11.4
2560	214.0	5.0	11.0
Yield, % of sugar	43	66	69

Medium, g./l.: beet molasses, 20; $(NH_4)_2HPO_4$, 1. Aerated during fermentation.

biotin content of *S. cerevisiae* increased progressivly 400-fold whereas the increases for *T. utilis* and *C. arborea* yeasts were only 2- and 30-fold, respectively. To a considerable degree, this difference reflects the needs of the cells for added biotin. Biotin is a growth factor for *S. cerevisiae*, but *T. utilis* and *C. arborea* can synthesize all the biotin needed for growth.

Other Vitamins

The data in the literature for choline and inositol are rather meager. One report for bakers' yeast *(1)* gives the value as 2100 mcg. for choline and 4320 mcg. for inositol per gram of dry matter. For brewers' yeast *(14)* the figures are 4850 mcg. for choline and 5000 mcg. for inositol. For primary-grown yeast *(3)* the values are 4000 mcg. of choline and 4000 mcg. of inositol. An average of 2,700 mcg. per gram of inositol has been reported for *T. utilis (16)*. Other vitamins, as yet unidentified, will undoubtedly be found in yeast.

Effect of Yield on Vitamin Content

It has been pointed out that the vitamin content of the yeast depends in many cases on the amount of these vitamins in the medium. The number of yeast cells may also influence the quantity of certain nutrients incorporated into the cell. Table 5 contains the results of such studies.

TABLE 5

Effect of Yield (Aeration) on Vitamin Content (21)

Yield on sugar fermented	Vitamins, mcg./g. dry matter			
	Thiamine	Riboflavin	Niacin	Folic acid
%				
S. cerevisiae				
20	53	91	210	26
31	41	67	323	34
42	35	56	439	22
T. utilis				
8	60	105	230	19
22	51	97	288	14
41	31	96	302	17
66	17	63	475	8
C. arborea				
8	43	95	316	41
20	30	83	316	22
32	21	70	326	17
75	13	56	376	17

Medium, g. l.: beet molasses 20; corn steep liquor 6.6; $(NH_4)_2HPO_4$ 0.5. Yield was regulated by the amount of aeration and agitation.

The yield of yeast was controlled by varying the aeration. All other conditions, such as medium, pH, etc., remained unchanged. It is seen that the thiamine content for all three yeasts dropped as the yield of yeast increased. Riboflavin and folic acid likewise declined. Niacin, however, rose markedly, particularly with *S. cerevisiae* and *T. utilis*.

Mineral Content of Yeast

Table 6 shows the meager data in the literature on the mineral content of yeast. Only two complete reports have appeared in recent years, those of Frey *(30)* and Garrod *(31)*. The elements for which the best data are available are calcium, phosphorus and iron. For comparison,

TABLE 6

Average Mineral Content of Yeast

(% dry matter)

Yeast	Ca	Mg	K	Na	Fe	P	S	Ref.
Bakers' *	0.038	0.145	2.23	0.149	0.017	1.62	0.015	(1)
Brewers' **	0.070	.....	.....	.....	0.009	1.52	.....	(28)
T. utilis	0.47	.....	.....	.....	.....	1.93	.....	(19)
Soybean flour	0.300	0.250	.....	.....	0.015	0.700	0.337	(7, 29)

*Cu 0.0021, Zn 0.013.
**Cu 0.004, Zn 0.005, Mn 0.0005, Co 0.0005.

figures for soybean flour *(29)* are included. Yeast resembles soybean flour rather closely in mineral content, particularly in iron and phosphorus. The calcium figure for *T. utilis* is for a sample grown in sulfite-waste liquor and appears very high in comparison with those for bakers' and brewers' yeasts. It should be checked with samples of *T. utilis* grown on other raw materials. Yeast is conspicuously rich in iron and phosphorus and would be a good source of these elements if included in the diet.

Summary

Yeast varies greatly in its content of B-vitamins and hence no single figures can be set to express the values. These variations depend somewhat on species of yeast but apparently more on composition of medium and other conditions of growth. The thiamine, niacin and biotin content of yeast can easily be increased twofold by adding these vitamins to the medium. The response to additions of the other vitamins appears to be much less marked. Yeast is a superior source of phosphorus and iron and compares favorably with many staple foods in calcium content.

REFERENCES

1. Frey, C. N. Personal communication.
2. Burkholder, P. R., MacVeigh, I., and Wilson, K. *Arch. Biochem.* **7**, 287 (1945).
3. Rudert, F. J. Personal communication.
4. Sarma, P. S., Snell, E. E., and Elvehjem, C. A. *J. Nutrition* 33, 121 (1947).
5. Hopkins, R. H., and Pennington, R. J. *Biochem. J.* **41**, 110 (1947).
6. Schwarz, R., Laufer, S., Laufer, L., and Brenner, M. W. *Ind. Eng. Chem.* **34**, 480 (1942).
7. Tables of Food Composition, U. S. D. A. Miscellaneous Publication No. 572 (1945).
8. Lampen, J. O., Baldwin, H. L., and Peterson, W. H. *Arch. Biochem.* **7**, 277 (1945).
9. Peterson, W. H., and Associates. Unpublished data.
10. Just, F. *Wochenschr. Brau* **57**, 233 (1940) also in Skoog Report (see Ref. 11).
11. Lundin, H. In Skoog report entitled "Food Yeast Production and Utilization in Germany," U. S. Dept. Commerce, Office of Technical Services P. B. 2041. Also in Wood Yeast for Animal Feed. Northeastern Wood Utilization Council. *Bull.* 12 (1946).
12. Food Yeast. A Venture in Practical Nutrition. Colonial Office, London, England (1944).
13. Brenner, Sadie. Personal communication.
14. Hoogerheide, J. C. Personal communication.
15. Rubin, S. H., Schreiner, J., and Hirschberg, E. *J. Biol. Chem.* **167**, 599 (1947).
16. Lewis, J. C., Stubbs, J. J., and Noble, W. M. *Arch. Biochem.* **4**, 392 (1944); also *Food Industries* **16**, 694 (1944).
17. Sreenivasan, A., Harper, A. E., and Elvehjem, C. A. *J. Biol. Chem.* **177**, 117 (1949).
18. Prouty, C. C. Wash. Agr. Exp. Sta. *Bull.* 484 (1947).
19. Wiley, A. J. Personal communication.
20. Agarwal, P. N., Singh, K., King, P. S., and Peterson, W. H. *Arch. Biochem.* **14**, 105 (1947).
21. Singh, K., Agarwal, P. N., and Peterson, W. H. *Arch. Biochem.* **18**, 181 (1948).
22. Agarwala, S. C., and Peterson, W. H. *Arch. Biochem.* In press.
23. Chang, W. S., and Peterson, W. H. *J. Bact.* **58**, 33 (1949).
24. Kurth, E. F., and Cheldelin, V. H. *Ind. Eng. Chem.* **38**, 617 (1946).
25. Van Lanen, J. M., Broquist, H. P., Johnson, M. J., Baldwin, I. L., and Peterson, W. H. *Ind. Eng. Chem.* **34**, 1244 (1942).
26. Van Lanen, J. M. *Arch. Biochem.* **12**, 101 (1947).
27. Holden, J. T., Furman, C., and Snell, E. E. *J. Biol. Chem.* **178**, 789 (1949).
28. Singleton, Elsie. Brewers' Yeast Council, Inc. Personal communication.
29. Sherman, H. C. Chemistry of Food and Nutrition, 6th Edition. The MacMillan Co., New York.
30. Frey, C. N. *Ind. Eng. Chem.* **22**, 1154 (1930).
31. Garrod, L. P. *J. Hyg.* **35**, 219 (1935).

Vitamin Availability

HELEN T. PARSONS
Department of Home Economics
University of Wisconsin

When unfortified fresh bakers' yeast was fed to human subjects, there was no increase in the urinary output of thiamine but a noticeable increase in the fecal thiamine *(1-7)*, indicating that the thiamine of the live yeast cell was not absorbed but was lost in the feces. No evidence was found that this might be due to an increased peristalsis *(2, 7, 8)* or to the phosphorylating ability of yeast *(4)*.

This peculiar behavior of the thiamine of fresh unfortified yeasts in contrast to that from other foods has been attributed in our laboratory to the nature of the cell membrane of live yeast. This withstands the action of the enzymes of the human digestive tract (a fact established by the finding of living yeast cells in feces after the feeding of live yeasts) *(3, 9, 10)* and hence opposes the loss of its cell nutrients. That this is a reasonable hypothesis was borne out by the results of further experiments. Treatments of the yeast cell which resulted in the death of the cell and a consequent loss of the selective permeability of the cell membrane rendered the thiamine of the cell as readily available to human utilization as that from other foods. Such effective treatments included suspending the yeast in 70 percent ethanol *(9)* or in boiling water and also the manufacturing processes in the preparation of commercial nutritional dried yeast *(1, 3, 7)*. On the other hand, bakers' yeast which had been frozen for short periods or which was dried without important loss of viability, as for example, active dried yeast for use in breadmaking, was found largely to retain its resistance to digestion and to the absorption of its thiamine by the human digestive tract.

As would be expected from the results on dead cells of bakers' yeasts, the thiamine in brewers' dried yeast tends to be highly available *(9, 11, 12.)* Sure tested 3 dried brewers' yeasts by feeding them to rats at levels as high as 10-15 times the requirement of the rat for thiamine *(12)*. With only one yeast did the increase in fecal thiamine above that due to a comparable dose of pure vitamin (6 to 12 percent more) indicate a relative failure of absorption. In growth experiments with rats and in

human urinary output studies in this laboratory, a debittered brewers' dried yeast was as well utilized as synthetic thiamine.

The performance of the rat in regard to growth on live yeast supplements has points both of agreement and disagreement with the evidence of low availability of thiamine to human subjects noted above. The first observation along this line was made by Walker and Nelson in 1933 *(13)* and has since been abundantly confirmed, i.e., the minimum dosage level of dead yeast that fully supplies the thiamine needs of a rat is entirely inadequate for this purpose if fed in the form of fresh live cells *(14)*. However, it is to be noted that rat growth tests fail to give a complete evaluation of the availability of yeast thiamine to human subjects inasmuch as any considerable increase in rat-dose of yeast above this minimum level furnishes the rat with a satisfactory quota of thiamine for growth even when fresh yeast is used *(11)*, whereas comparable increases in fresh yeast doses for human subjects do not increase the output of urinary thiamine. Evidently the human digestive tract finds more difficulty in digesting live yeast than does the digestive tract of the rat. This is confirmed by the contrast between the two types of subjects when live dried yeast is fed; most rats have little difficulty in deriving a satisfactory growth quota of thiamine from even minimal levels of this type of live yeast *(11)* whereas the thiamine from it is unavailable to human subjects *(7)*. This emphasizes the necessity of carrying out specific tests on human subjects in order to draw conclusions that are to be applied to the feeding of human beings.

A further peculiar effect of live yeast in the human digestive tract relates to the observation *(3, 5, 7)* that live yeast cells actually interfere with the absorption of thiamine both from other foods and from doses of pure thiamine hydrochloride in the digestive tract. Within the limits of experiments in this department, the larger the dose of live yeast ingested by human subjects and the longer the dose was fed, the greater was the decrease in urinary thiamine in comparison with the output on the basal diet alone and the slower was the return to the basal level of urinary thiamine. This interference with food thiamine was somewhat lessened in the active yeasts dried for use in breadmaking without serious loss of viability, and abolished by processes which destroyed viability of the yeast cell *(7)*. That the low urinary return of the food thiamine was due to lack of its absorption from the digestive tract and not merely to a relative lack of excretion was proved by the demonstration that both young growing pigs and rats, fed live bakers' yeast stored

only about half as much thiamine in their body tissues as those on the basal diet alone. Thus, urinary output was an index of the amount absorbed under these conditions *(11, 14, 15)*.

A reasonable hypothesis to account for these observations is that, inasmuch as live yeast is known to avidly absorb thiamine from surrounding media, it competes with the absorbing membranes of the human digestive tract for available thiamine in the food mixture. An analogy is seen in the behavior of yeast in the fermentation of wort, cited by Levine *(16)*; the active yeast cells absorb a large part of the thiamine, but leave the riboflavin mainly in the beer. The analogy is the more perfect in that experiments with human subjects have shown that food riboflavin in the digestive tract is not interfered with by fresh yeast although the riboflavin within the live yeast cell itself is largely unavailable *(6, 9)*.

In further experiments with human subjects, variations were made in the distribution throughout the day of doses of pure thiamine hydrochloride and also of supplements of yeast, especially with respect to meal time. In agreement with Melnick, *et al. (17)* it was found that doses of thiamine hydrochloride at meals gave about two times the amount of increase in urinary thiamine returns that was given by the same dose between meals, an effect not completely understood. However, whether fresh bakers' yeast accompanied the thiamine hydrochloride at meal time or between meals, the effect was always to tend to hold back the return of the thiamine dose in the urine and hence, by inference, to interfere with the absorption of the dose of thiamine hydrochloride.

Inasmuch as the pure vitamin as well as food thiamine is interfered with by live yeast, one conclusion drawn from this experiment is that the effect of fresh yeast on thiamine in the digestive tract is not analogous to the effect on folic acid, claimed for certain yeast extracts, i.e., a blocking of a conjugase that would otherwise free the vitamin for absorption *(9)*.

It is of great theoretical interest that human subjects for whom the thiamine of live yeast is unavailable have been found, in our laboratory *(9)* to make a very good use of the protein of this active yeast. The relative availability of the nitrogen of fresh yeast is not necessarily inconsistent with the finding of live yeast cells in the feces as these ordinarily represent only about 1 to 2 percent of the number ingested. Further research will be necessary in order to make clear why a delay

in the death of live yeast cells in the human digestive tract is associated with so outstandingly greater an absorption of the protein of the cell than of its thiamine. A barrier mechanism in the digestive tract has been postulated *(18)* to account for the relatively limited absorbability of thiamine by human subjects. The possibility of a relation of such a mechanism to the effect of fresh bakers' yeast is being explored.

Utilization of Riboflavin in Yeasts

The experiments on riboflavin may be summarized as follows *(1, 6)*: Riboflavin in live fresh unfortified bakers' yeasts gives little or no evidence of being absorbed by the human digestive tract. Riboflavin added by fortification is fully utilized by human subjects and is probably not absorbed into the yeast cell at all. Live dried bakers' yeast for bread-making gives up a part of its riboflavin (though not its thiamine) for absorption. Riboflavin is fully available in bakers' yeasts in which viability is destroyed by manufacture of nutritional dried yeast, or by suspension in 70 percent ethanol or in boiling water. Live bakers' yeast does not absorb riboflavin from other foods in the digestive tract, an observation in harmony with the behavior of yeast in fermenting wort. The distribution of doses of riboflavin in respect to meals has no measurable effect on its absorption and return in the urine.

Utilization of Biotin From Yeast

Gardner *et al. (19)* fed human subjects comparable amounts of biotin in the forms of fresh bakers' yeast and of beef liver and found a much larger urinary output from beef liver, hence, presumably a greater availability of biotin in this food than in fresh bakers' yeast. Moreover, it appears to be more difficult to increase the availability of biotin than of thiamine or of riboflavin in bakers' yeast by various treatments. Suspension in boiling water which releases both thiamine and riboflavin in yeast for absorption had no measurable effect in increasing the availability of the biotin. Autolysis is known to completely liberate the thiamine, riboflavin and nicotinic acid from brewers' yeast cells in as short a time as 2¾ to 3 hours *(20)*. However, when human subjects ingested bakers' yeast which had been autolyzed for 2 hours and then suspended in boiling water for 1 hour, the urinary output was still strikingly low in comparison with that from a comparable amount of biotin in liver *(19)*. Similar experiments are not yet completed on brewers' and other types of yeast.

Utilization of Folic Acid From Yeasts

There is evidence that certain conjugates of folic acid injected in the pure form or fed as dried brewers' yeast are unavailable to the mink and fox *(21, 22, 23)*. Similar tests with human subjects are in progress.

Other B Vitamins in Yeasts

The literature on the nutritive value of food yeasts and the capacity of these yeasts to relieve specific signs of vitamin deficiency has been well reviewed *(24, 25)* and indicates considerable availability of both recognized and unidentified vitamins. However, the practical question of exactly how much of each vitamin can be derived by human subjects from the relatively limited amounts of food yeasts which people in general seem to find it acceptable to consume day in day out in their food, becomes a matter of exact quantitative experimentation not only on the content of each factor in yeasts but also their quantitative availability. Very few experiments of the kind have been reported. Such studies are now in progress.

Summary

The literature on the nutritive value of food yeasts and the capacity of these yeasts to relieve specific signs of vitamin deficiency in human beings and animals indicates considerable availability of recognized and unidentified vitamins in these yeasts.

However, evidence shows that thiamine, riboflavin and biotin of active bakers' yeast are not readily available. Moreover, the thiamine of food is absorbed and stored in the tissues less well if active bakers' yeast is also eaten. This may be because live yeast avidly absorbs thiamine and would therefore presumably compete with the digestive tract. Alternate hypotheses have also been suggested.

Drying, freezing and other processes which injure the vitality of the yeast cell render the thiamine and riboflavin available. Thus food yeasts which are non-active are very available sources of these vitamins. The effect of such processes on the availability of other B-vitamins as niacin, biotin and folic acid is still under investigation.

REFERENCES

1. Parsons, H. T., and Collord, J. *J. Am. Dietet. Assoc.* **18**, 805 (1942).
2. Parsons, H. T., Stettler, H., Williamson, A., and Johnson, M. L. *J. Canad. Dietet. Assoc.* **6**, 43 (1944).

3. Parsons, H. T., Williamson, A., and Johnson, M. L. *J. Nutrition* **29**, 373 (1945).
4. Hochberg, M., Melnick, D., and Oser, B. L. *J. Nutrition* **30**, 201 (1945).
5. Ness, H. T., Price, E. L., and Parsons, H. T. *Science* **103**, 198 (1946).
6. Price, E. L., Marquette, M. M., and Parsons, H. T. *J. Nutrition* **34**, 311 (1947).
7. Kingsley, H. N., and Parsons, H. T. *J. Nutrition* **34**, 321 (1947).
8. Russell, R. A., and Nassett, E. S. *J. Nutrition* **22**, 287 (1941).
9. Garber, M., Marquette, M. M., and Parsons, H. T. *J. Nutrition* **38**, 225 (1949).
10. Fung, Fung-haan, Aaness, R., and Parsons, H. T. *Wisconsin Academy of Sciences, Arts and Letters,* Stevens Point, Wisconsin. April 24, 1948.
11. Unpublished data.
12. Sure, B. *Arch. Biochem.* **4**, 413 (1944).
13. Walker, R., and Nelson, E. M. *Am. J. Physiol.* **103**, 25 (1933).
14. Parsons, H. T., Foeste, A., and Gilberg, H. *J. Nutrition* **29**, 383 (1945).
15. Fung, Fung-haan, Aaness, R. J., and Parsons, H. T. *Fed. Proc.* **8**, 383 (1949).
16. Levine, H. *Proc. Soc. Brewing Chem.* **4**, 43 (1941).
17. Melnick, D., Field, H., and Robinson, W. D. *J. Nutrition* **18**, 593 (1939).
18. Friedemann, T. E., Kmieciak, T. C., Keegan, P. K., and Sheft, B. B. *Gastroenterology* **11**, 100 (1948).
19. Gardner, J., Parsons, H. T., and Peterson, W. H. *Am. J. Med. Sci.* **211**, 198 (1946).
20. Farker, K. T. H. *Australian J. Exper. Biol. and Med. Sci.* **24**, 213 (1946).
21. Schaefer, A. E., Whitehair, C. K., and Elvehjem, C. A. *J. Nutrition* **35**, 147 (1948).
22. Schaefer, A. E., Tove, S. B., Whitehair, C. K., and Elvehjem, C. A. *J. Nutrition* **35**, 157 (1948).
23. Tove, S. B., Schaefer, A. E., and Elvehjem, C. A. *J. Nutrition* **38**, 469 (1949).
24. Prouty, C. C. Brewers' yeast in human and livestock nutrition. A review of the literature. *Bull. No.* **484**, State College of Washington, Institute of Agricultural Sciences, Agricultural Experiment Stations, Pullman, Washington. Jan. 1947.
25. von Loesecke, H. W. *J. Am. Dietet. Assoc.* **22**, 485 (1946).

Protein Values of Yeast

PAUL R. CANNON [1]
Department of Pathology
The University of Chicago

The Rat-Repletion Method

The protein-values of yeasts as single proteins as well as their supplementary value to white flour were determined by the rat-repletion method.[2]

The rat-repletion method *(1)* was developed during the war. In principle, it consists in the production of a moderately severe degree of protein deficiency in young adult male white rats and then in determining the capacity of these depleted animals to recover lost weight, lost plasma proteins, etc., after feeding a basal ration adequate in calories, vitamins, and salts and containing 9 percent of the protein to be assayed. By using a standard high-quality protein under identical conditions it is possible to compare the performance of groups of animals in relation to the standard. The rate of tissue repletion is actually a measure of the extent of overall tissue protein synthesis accomplished by the test protein. For example, groups of depleted rats fed the repletion ration containing a high quality protein (lactalbumin-casein mixture) for 14 days will regain approximately 65-70 grams of lost weight. This represents mainly regeneration of muscle mass, liver protein, enzyme systems, etc. The repletion ration as offered in 15-gram daily portions supplies 48 calories per rat per day, and a surplus of all the known vitamins needed by the rat. With this method one can assay not only protein, but amino acid mixtures as well. Thus it is possible to substitute a mixture of sixteen crystalline amino acids, compounded in the proportions in which these occur in casein, for natural protein and feed a ration containing these amino acids to depleted rats. Such animals in ten days will recover from 40 to 50 grams of lost weight, or approximately 4.5 grams per day. However, if the quantity of any one of nine essential amino acids is reduced below the daily requirement for the depleted rat, the animals will fail to recover lost weight. This evidently means that, in the evaluation

[1] The following collaborated in the experimental work: L. E. Frazier, R. W. Wissler, E. P. Benditt, E. M. Humphreys, C. H. Steffee and R. L. Woolridge.

[2] This work was done in cooperation with the Quartermaster Food & Container Institute for the Armed Forces.

of a protein, the limiting action of any essential amino acid in the protein will affect its nutritive value, assuming that the protein is well digested and absorbed. Under such conditions, therefore, the rat-repletion method measures essentially the amino acid value of the protein being tested.

The Evaluation of Yeast-Protein By the Rat-Repletion Method

When dried yeasts were tested under these conditions the values were as follows: With hydrolyzed yeast the rats regained an average of 46 grams of weight; with dried brewers' yeast an average of 42 grams; with hydrolyzed casein, 54 grams; and with lactalbumin, 65 grams. It is obvious from these comparative values that the yeast protein was of excellent quality, even though somewhat inferior to lactalbumin and casein. It should be added, moreover, that under similar conditions yeast protein exceeds the values found for soy protein, sunflower seed protein, peanut protein, navy beans, and peas. The limiting amino acid in all these vegetable proteins is presumably methionine, according to chemical and biological assays.

The Evaluation of the Protein Value of Bread After Supplementation With Yeast

Since the lysine content of yeast is fairly high, yeast might be especially useful in supplementation of the defective proteins of white flour. Experiments were undertaken therefore to study the effects of yeast supplementation on the nutritive value of canned bread. Three canned breads were prepared at the QM Subsistence Research & Development Laboratory [3] from the following basic ingredients: (1) white flour plus 2 percent milk solids and the usual enrichment synthetics, (2) white flour plus 2 percent milk solids plus 3 percent dried brewers' yeast [4] fortified with synthetics to equal the usual enrichment levels, and (3) whole wheat flour plus 2 percent milk solids. The breads prepared from these mixtures were ground and air-dried. After proximate analyses had been made these three types of breads were incorporated into the basal ration at the same protein concentration and fed in the usual way to groups of protein-depleted adult white rats. As a control, two additional groups of rats were fed the identical rations with lactocasein as the only source of protein. Furthermore, in one group of these latter animals food consumption was restricted to the amount of ration eaten

[3] Now known as Quartermaster Food & Container Institute for the Armed Forces.

[4] This yeast was supplied by Haffenreffer Yeast Co., Jamaica Plains, Mass.

by the rats supplied with the enriched white bread. The breads will be referred to hereafter as (1) white bread, (2) yeast white bread, and (3) whole wheat bread. The results are shown in Table 1.

Comment: It can be seen from the table that the addition of 3 percent of dried brewers' yeast to the white bread improved its nutritive potentiality considerably, making it practically equal, in fact, to the whole wheat bread. This conclusion is justified whether based on the average weight gains for the two-weeks period, or on the basis of protein efficiency (grams gained per gram of protein eaten). If one considers the enriched white bread figures as a baseline, the animals eating the yeast bread

TABLE 1

*Results for Fourteen-Day Feeding Period**

Type of Protein and Rat Number	Grams of Protein Consumed	Weight Gain Grams	Ratio of Grams of Weight Gained per Gram of Protein Consumed
White Bread			
243-3	17.0	21	1.24
247-3	16.1	21	1.26
248-3	16.2	20	1.23
248-4	17.1	19	1.11
250-4	17.9	23	1.29
250-6	17.1	27	1.58
252-1	17.8	27	1.52
254-1	17.3	20	1.16
254-5	16.4	24	1.46
256-1	18.3	20	1.09
256-7	16.0	23	1.44
Av.	16.1	22	1.31
Yeast White Bread			
247-2	18.8	27	1.43
248-7	18.8	30	1.59
249-2	18.3	26	1.42
250-3	17.9	27	1.51
251-4	18.3	31	1.69
252-2	19.5	29	1.48
254-3	17.4	26	1.50
254-4	17.6	29	1.64
254-6	17.9	32	1.78
255-5	18.4	33	1.79
256-5	18.6	27	1.39
Av.	18.3	29	1.57

TABLE 1 *(Continued)*

Type of Protein and Rat Number	Grams of Protein Consumed	Weight Gain Grams	Ratio of Grams of Weight Gained per Gram of Protein Consumed
Whole Wheat Bread			
245-6	17.6	20	1.14
246-6	18.6	36	1.93
247-6	18.6	28	1.50
249-4	19.1	28	1.46
250-5	17.3	29	1.68
251-2	18.9	30	1.58
251-7	18.5	23	1.24
252-5	19.4	32	1.65
253-1	18.9	23	1.22
253-2	18.7	31	1.66
255-6	19.2	33	1.72
Av.	18.6	28	1.53
Lactocasein			
243-7	21.7	69	3.17
251-5	21.6	68	3.14
256-4	21.8	67	3.07
256-6	21.5	74	3.44
Av.	21.7	70	3.21
Lactocasein Limited			
243-2	17.5	59	3.36
245-5	17.7	54	3.06
246-5	17.5	54	3.08
249-7	17.7	62	3.51
Av.	17.6	57	3.25

* Actual protein percentages of the rations were as follows: White bread, 9.07; Yeast bread, 9.16; Whole Wheat bread, 9.11; Lactocasein, 9.75.

showed an average increase in weight recovery of approximately 33 percent, and at least a 20 percent improvement on the basis of grams gained per gram of protein eaten. The figures for the animals eating the whole wheat bread give practically the same indication of superiority over the enriched white bread alone.

If one compares the performance of these three groups of animals, however, with that of the two smaller groups eating lactocasein as their source of protein one can see the superior nutritive value of the high quality protein of lactocasein. On the basis of grams gained per gram

of protein eaten the protein efficiency of the white bread is only about 40 percent of that of the lactocasein. On the same basis, the protein efficiency of the yeast bread and the whole wheat bread is approximately 48 percent.

It should be noted that in addition to the vitamins added to these samples, the basal ration also contains adequate quantities of oleum percomorphum, calcium pantothenate, pyridoxine, riboflavin, thiamine, niacin, and choline, as well as liver concentrate. The results, therefore, are interpreted as expressing, for the most part, the supplemental action of amino acid enrichment from the yeast.

In conclusion, these findings indicate that (1) The enriched white bread showed between a 20 and 33 percent nutritive improvement as the result of supplementation with 3 percent dried brewers' yeast, practically equaling, in fact, the nutritive value of the whole wheat bread. (2) The protein efficiency of the enriched white bread was approximately 40 percent of that of lactocasein fed in a ration in comparable amounts. The protein efficiency of the whole wheat bread and of the yeast-supplemented white bread was approximately 48 percent of the efficiency of lactocasein. (3) It is obvious that in addition to the contribution of vitamins, yeast supplementation of white bread improves its protein value materially as tested under the conditions of these experiments. Because yeast contains all the essential amino acids in good amounts there is no good reason from the nutritive standpoint why it should not be used more generally for further enrichment of white flour. If the protein value of white flour can be restored to that of whole wheat flour, perhaps much of the old controversy of white versus brown bread is no longer necessary and it should be possible to have a white bread without the loss of important nutritive qualities due to processing methods.

REFERENCES

1. Wissler, R. W., Woolridge, R. L., Steffee, C. H., and Cannon, P. R. *J. Immunol.* **52**, 267 (1946).

Composition of Brewers' Yeast Extracts

B. L. MAIZEL
Vico Products Co., Chicago, Illinois

Surplus brewers' yeast may be efficiently utilized in human nutrition by converting it to autolyzed yeast extracts. The products obtained on autolysis are high in protein, rich in B-complex vitamins, and may be modified in several ways to permit a variety of uses. The scope of usefulness of yeast extracts can best be made clear by describing them in terms of the method used in their manufacture and the available analytical data.

The process of autolysis of brewers' yeast is very simple. The yeast is brought from the breweries as a 12 to 20 percent suspension, and is stored, until used, in cooolers at about 40° F. When kept at normal beer cellar temperature, 40° F., the yeast remains active and viable for about two weeks after harvesting. For this reason, it is possible to utilize yeast even from the smaller breweries, where yeast may be collected only every other week. The solid particles of hop resin are removed by passing the yeast through a 100 to 120 mesh screen, and the beer is washed out by diluting the suspension and removing the excess liquid by any of several methods. Hop resins adhering to cell walls are solubilized by raising the pH to about 6.5 or 7 with sodium carbonate. The dissolved resin is eliminated by repeated washings which may be carried out either on filter presses or with the aid of standard yeast separators. With some types of brewers' yeast efficient washing can be achieved by dilution and decantation. All three washing processes are used by various producers on a commercial scale.

It is very important to keep most of the cells alive during the washing processes in order to retain maximum nutrient content. The presence of dead yeast cells reduces the efficiency of extraction, since some of the desirable constituents diffuse through the cell membrane. Live, active cells can be put through washing operations with water and alkali without loss of vitamin, protein, or enzyme activity.

The autolysis of washed yeast is initiated by raising the temperature of the suspension above 100° F. It appears that enzymes active in the autolysis of yeast are more efficient in the washed yeast than in the

presence of beer or hop resins. The operation is more rapid and a greater digestion of protein, as indicated by formol titration, is obtained, if the yeast suspension is made hypertonic with sodium chloride or if an organic fat solvent, such as ethyl acetate, is added to the suspension.

Autolyzed extracts containing salt are mostly used in the food industries while the salt-free autolyzed yeast extracts have greater use in the pharmaceutical field. It is interesting to note that the removal of hop resin flavors from brewers' yeast presents no problems.

Various vitamins and protein factors diffuse from the cell at different rates. Thiamine diffuses from the cell very rapidly. The nitrogen fraction, which appears in the solution during the first hour, has an extremely high formol titration. As the total nitrogen in solution increases, the formol titration drops. The diffusion of riboflavin is approximately parallel to that of the protein. Upon completion of the autolysis, the cell membranes are separated by filtration or centrifugation. The use of a filter press permits recovery of a more concentrated residue and reduces the time required for the evaporation of the extract; the use of a centrifuge requires less direct labor per unit weight of yeast. The extract is then concentrated in a vacuum evaporator to a thick paste of about 75 percent solids or diluted to 40 percent solids and spray-dried or vacuum shelf-dried. The final operation depends upon the nature of the end product desired.

The efficiency of the extraction is controlled by running nitrogen and vitamin assays on the diluted suspension and on the filtrate of that suspension. When the process is carried out properly, the figures obtained for the suspension and the filtrate are identical within experimental error, indicating that complete diffusion of these various factors has occurred. Of course, this does not establish that the distribution of amino acids and of various vitamin B-complex factors in the autolysate is identical with the distribution of these factors in the original yeast. (See Table 1)

It is interesting to note that the total thiamine value of fresh brewers' yeast as determined by the thiochrome technique will vary from 60 to 150 micrograms per gram on a dry basis. The thiamine values of yeasts which initially show a low figure, however, tend to approach the maximum of 120 to 150 micrograms per gram on a dry basis upon autolysis or on prolonged aging at room temperature, under conditions which do not show any evidence of autolysis. A possible clue to this behavior lies in the fact that cocarboxylase of brewers' yeast does not occur in a free

TABLE 1

Concentration of Vitamin B-Complex Factors in Dry, Salt-Free Autolyzed Brewers' Yeast Extract

	Potency per gram
Thiamine	0.26 mg.
Riboflavin	0.08 mg.
Pyridoxine	0.06 mg.
Niacin	0.65 mg.
Calcium Pantothenate	0.12 mg.
Choline	10.00 mg.
Folic Acid	28.00 mcg.
Biotin	3.00 mcg.
Inositol	8.00 mcg.
Streptogenin	2 units

state but exists as a fairly stable complex which is not readily broken to liberate all the thiamine by the standard method of extraction. Further evidence that the present method of determination of thiamine in yeast does not give a maximum figure is indicated by the fact that the extraction of yeast with hot water gives a figure which is consistently about 10 percent higher than that obtained when a standard acid extraction procedure is followed.

An examination of the distribution of nitrogen bonding present in autolyzed brewers' yeast extract (Table 2) shows that the purine nitrogen is fairly low, representing less than 5 percent of the total nitro-

TABLE 2

Distribution of Nitrogen in Dry, Salt-Free Autolyzed Brewers' Yeast Extract

Total Nitrogen	11.2%
Amino Nitrogen (Sorenson Method)	3.9%
Ammonia Nitrogen	0.08%
Purine Base Nitrogen	0.62%

gen present. The ammonia nitrogen is less than 1 percent of the total nitrogen present. This low ammonia nitrogen does not necessarily indicate that deamination has not occurred since some ammonia may be lost during evaporation and drying. The distribution of amino acids in the autolysate, however, is similar to the accepted values for yeast (Table 3) showing that no appreciable deamination of any single acid has occurred.

TABLE 3

Distribution of Certain Amino Acids in Dry Autolyzed Brewers' Yeast Extract

Calculated to 16% N basis

Arginine	6.3%	Lysine	6.0%
Cystine	0.9%	Methionine	2.0%
Glutamic Acid	10.8%	Phenylalanine	4.3%
Glycine	4.9%	Threonine	2.7%
Histidine	3.2%	Tryptophane	0.9%
Isoleucine	4.7%	Tyrosine	1.6%
Leucine	6.3%	Valine	4.7%

The nutritional qualities of autolyzed yeast extract protein were evaluated by a series of rat growth tests. These tests were made to determine what modifications are necessary to obtain the maximal rate of growth when the yeast extract is used as the sole source of nitrogen at a 1.2 percent level.

Four-week feeding tests were performed on rats weighing approximately 45 grams at the beginning of the tests. Ten rats were employed on each level. The diet was fed ad libitum and consisted of: lard, 9.0%; salt, 4.0%; cod liver oil, 2.0%; wheat germ oil, 1.0%; brewers' yeast powder, 2.0%; thiamine chloride, 0.0006%; riboflavin, 0.0002%; and starch, to make 100%.

The protein fraction consisted either of yeast hydrolysate or of yeast hydrolysate supplemented with several levels of cystine and methionine. In these tests, the source of protein is considered satisfactory if the rats gain an average of 36 grams in four weeks. The gain per gram of nitrogen fed on this type of yeast extract is lower than that obtained on a normal stock diet, but the test is designed to accentuate the differences between various proteins rather than to obtain the greatest gain. It is interesting to note (Table 4) that while increasing quantities of cystine and methionine fed separately did not produce appreciable increases in the total weight gain or the gain per gram of nitrogen consumed, either of these amino acids when fed at levels exceeding 0.5 percent produced more uniform growth results than the 0.5 percent level as judged from the decreases in the percentage deviation from the mean. However, when both cystine and methionine were included in the diet simultaneously, each at a 0.5 percent level, the weight gain increased to 47.5 grams as compared to 33.4 grams and 25.4 grams for cystine and methionine respectively, when either one is fed singly at this level. Similar findings were observed for the weight gain per gram of nitrogen consumed. The percentage deviation from the mean of the weight gain remained low.

TABLE 4

Evaluation of Dry Autolyzed Yeast Extract Protein

Composition of Protein Supplement	AVERAGE WEIGHT GAIN FOR 4 WEEK PERIOD		AVERAGE FOOD CONSUMPTION FOR 4 WEEK PERIOD		
	Gain in Grams	Percent Deviation from Mean	Consumption in Grams	Percent Deviation from Mean	Weight gain per gram of nitrogen consumed
Autolyzed Yeast Extract	20.0	24.0	242	1.73	6.9
Autolyzed Yeast Extract plus 0.5% Cystine	33.4	21.5	240	5.50	11.5
Autolyzed Yeast Extract plus 1% Cystine	35.7	6.40	241	1.90	12.3
Autolyzed Yeast Extract plus 2% Cystine	36.0	11.1	243	1.60	12.3
Autolyzed Yeast Extract plus 0.5% Methionine	25.4	33.5	231	7.70	9.1
Autolyzed Yeast Extract plus 0.7% Methionine	27.8	5.89	223	5.05	10.3
Autolyzed Yeast Extract plus 0.5% Cystine and 0.5% Methionine	47.5	14.3	248	6.81	15.9

The data presented demonstrate that autolyzed yeast extracts are nutritionally valuable because they are an extremely rich source of B-complex vitamins and have a high protein content with a desirable distribution of amino acids. Because of their meat-like flavor, yeast extracts have a place in certain manufactured meat products such as canned liver, potted meats, luncheon meats, meat loaves and products in which cereals are normally used. The flavor of prepared soup mixes, gravies and meat fillers also may be enriched by the addition of yeast extracts and several restaurant chains routinely use them in their soups, beef stews, meat loaves and similar dishes. Since autolyzed yeast apparently enhances the flavor of spices, it is important to reduce the quantity of condiments when yeast extracts are employed.

In baby foods yeast extracts impart a desirable flavor to products usually unspiced. Some experiments indicate that bland foods when flavored with yeast hydrolyzed protein are more pleasurable to young children.

Thus the use of autolyzed yeast extracts in food products permits desirable modification of flavor accompanied by the enrichment of the nutritional value of the finished material.

Experimentation With Dried Yeast for Use in Foods

SADIE BRENNER
9818 S. Normal, Chicago 28, Illinois

The importance of yeast as a possible enriching agent for foods was recognized and employed extensively by the Germans during both World Wars I and II *(1, 2, 3)*. In World War II it is estimated that they produced 100,000 tons annually *(4)*. The prominence of yeast in the military dietary was observed in captured rations where yeast was found in the form of liquids, pastes, syrups, and powders *(5)*.

Impetus to the use of yeast for human consumption was given in this country and Great Britain during the last war as a result of the impending food shortages *(2)*. In the post-war era yeast has been advocated as one of the major constituents of low cost food preparations for relief feeding *(6, 7, 8, 9, 10)*. As a result of these interests large quantities of brewers' yeast were salvaged for animal and human consumption.[1]

The dried yeast was intended not only as a supplement to low protein dietaries but also as a means of replacing some of the nutrient losses incurred in institutional food service due to the unavoidably long delay between preparation and consumption. Interest in yeast as a natural source of essential nutrients was expressed in the military feeding program *(11)* as a possible method of replacing heat-labile B complex vitamins partially destroyed during processing and storage.

The addition of yeast to foods present several problems, namely, those that derive from the possible impairment of flavor and appearance produced by changes in texture, color, and taste of the enriched product. The goal of yeast enrichment, therefore, should be not only the development of products in which yeast contributes significantly to the nutritive value but also products in which yeast enhances the acceptability or does not affect it.

---

[1] In the United States in 1945, 10,868,883 pounds brewers' yeast were dried, of which 2,509,281 pounds were debittered and used as nutritional yeast. Another 5,700,000 pounds were supplied as primary grown yeast *(4)*.

Civilian Application

Since the establishment of the flour and bread enrichment program, several investigations have been made employing yeast to replace some of the nutrilities lost in milling wheat flour *(12)*. McCollum suggested the use of 6 percent skim milk solids, wheat or corn germ, soy flour or dried brewers' yeast for the improvement of the nutritional quality of bread. Bread supplemented with these materials gave loaves of good palatability but did not score high by the conventional scoring method *(13, 14)*. Schwarz *et al. (15)* obtained poor quality breads when levels of 5 and 7½ percent of dried debittered brewers' yeast were used in flour but a 2½ percent level gave a loaf equal to the control except for a slightly reduced volume, and raised the thiamine, riboflavin, and niacin content of the bread above the tentative minimum requirements set up by the Food and Drug Administration for enriched white bread. These authors even suggest the use of irradiated brewers' yeast to supply vitamin D in bread.

Davis and Frenkel *(16)*, using a large number of both primary-grown and debittered brewers' yeasts, tested the effects of these yeasts on bread volume when added to flour at a 3 and 5 percent level. Only two of the yeasts gave bread of satisfactory volume at the 3 percent level. All the others showed a weakening of the gluten structure of the dough. This effect was satisfactorily overcome by proper treatment of the yeast prior to its incorporation into the dough. The treatment consists of oxidation or heating and oxidation. With this treatment the 5 percent level of yeast was found to be approaching the maximum which could be employed for bread enrichment.

Carr *(17)* working with dried brewers' yeast, and Sure *(18)* with primary-grown yeast found that from 6 to 15 percent yeast on the flour basis could be incorporated successfully into cakes, cookies, muffins, and other quick breads provided the dough was baked immediately after the yeast was added. The higher concentrations of yeast were used in the products containing molasses, spices and honey since those ingredients were capable of masking the yeast flavor.

In employing yeast in other foods Heller *(19, 20)* and Neidert *(21, 22)* worked out small quantity recipes first and then developed the large quantity recipe. Dried brewers' yeast was added to typical dishes: soups, meats, meat substitutes, vegetables, salads and salad dressings, sandwich fillings, hot cereals and bakery products. The amount of yeast which could be incorporated successfully varied with the food. In meat loaf and

LIBRARY

other prepared foods in which the flavor of onion and celery predominated or in baked products containing a considerable quantity of spice or chocolate, they found it possible to use 6 to 8 ounces (3 to 5 percent) of dried yeast for 50 portions. For creamed vegetables and creamed soups the addition of only 3 ounces (1 percent) for 50 portions was feasible. The yeast imparted a distinct flavor to some of the foods intensifying the meat flavor in meat dishes and adding a cheesy flavor to creamed dishes. When the yeast flavor was too pronounced bitter tastes were noted often as an after taste. In certain recipes, especially in those employing the larger amounts of yeast, it was desirable to decrease the salt content. In the sauce and gravy dishes the yeast was found to have a thickening effect thus making an increase in the liquid necessary. A dullness or greying of color of some enriched products was also noted but this could be camouflaged by various coloring agents, etc. Neidert reported that yeast did not blend well in flavor or color with tomatoes or tomato dishes, resulting in a less palatable and less attractive product. The addition of yeast to mayonnaise and French dressing was reported as not successful, the yeast causing the emulsion to break.

Sunderman *et al. (23)* successfully added dried primary-grown yeast at the rate of 3 ounces per gallon (2½ percent) to several cafeteria-served foods.[2]

Sure *(24)* confirmed the lack of detection of a 3 percent level added to various foods served in a college dormitory.[3]

Paul *et al. (25)* studied the effect of adding a dry primary yeast to corn meal and macaroni products. Experienced judges and a larger consumer panel rated 1 percent yeast by weight per cup of corn meal as generally acceptable but higher levels could be detected in corn meal products. In boiled macaroni a 2½ percent level was readily detected whereas in flavored macaroni dishes such as macaroni and cheese 4 and 7 percent levels were satisfactory. Feeding tests with these enriched materials showed that improvement in the nutritive value of the corn meal and macaroni was slight unless more than 3 percent yeast can be added *(26)*.

Roth and Zander *(27)* recommended the addition of one tablespoon of brewers' yeast to the daily dietary and suggested possible carriers for it

---

[2] Creole bean soup, chop suey, veal stew and macaroni and tomato sauce.

[3] Sure also reports that a 20 percent yeast-peanut butter mixture obtained very favorable acceptance among nursery school children.

such as peanut butter, malted milk, gravy, ketchup, etc. However, no mention is made of the acceptability of the resulting products.

Cognizance of the value of dried yeast as a supplement to the modern dietary has been given in a current popular cookbook where suggestions and directions for the addition of dried yeast to a large number of recipes are included *(28)*.

Dried yeast has been successfully incorporated into concentrated foods for convalescent and relief feeding. Spinella *(29)* prepared a high vitamin, high caloric sweetmeat containing 6½ percent brewers' yeast. Spies used a yeast-peanut butter mixture containing 25 percent brewers' yeast *(30)*. Several other types of food such as dehydrated soup mixes *(7)*, milk beverage bases, wafers, etc. have also been offered for sale *(31)*.

Studies on the utilization of yeast in commercial candy have indicated that at a 2 percent level the yeast flavor is not detected. Higher levels of yeast may be incorporated when other ingredients or artificial flavors are used to mask the yeast *(32)*.

Military Feeding *(33, 34)*

Yeast addition was attempted in several B ration and operational ration items. Bollman *(35)* added yeast to several representative components of the B ration, namely, canned luncheon meat, meat and beans, meat and vegetables, canned peas, and green beans. Both debittered brewers' and primary grown yeasts were employed at ⅓, 1, 3, and 5 percent levels, enriching the foods immediately prior to serving. The optimum level of yeast which could be used successfully varied with the particular yeast. Although levels up to 3 percent of some yeasts could not be detected in luncheon meat, levels over 1 percent for the other yeasts were rated unacceptable. In canned vegetables and canned meat and vegetable combinations ⅓ and 1 percent levels were found to be the most suitable.

The other investigations on yeast for use in Army feeding centers around a search for operational ration items which would supply and retain a significant portion of the B complex vitamins especially thiamine. The work to be described consists of the experiences encountered in the addition of yeast to various operational ration items. The general plan was to establish the optimum threshold of yeast for any particular product and then to continue to test the palatability of the enriched food and its

control following storage at various temperatures.[4] Retention of thiamine, riboflavin, and niacin was also measured at frequent intervals during storage.

Both primary grown and debittered brewers' yeasts[5] were added to biscuits at levels ranging from 1 to 3 percent of the weight of the flour. The 1½ percent level was satisfactory for the twenty yeasts tested, although for some yeasts higher levels could be employed. On storage at elevated temperatures for periods as long as 6 to 12 months the 1½ percent enriched biscuits retained comparable acceptability to the control samples. The biscuits through the yeast supplied approximately one-half of the daily thiamine requirements in the C, K, and 10-in-1 rations and also insured a supply of the lesser known vitamin factors *(36)*.

Primary grown and debittered brewers' yeasts were also added at 1 and 3 percent levels to canned bread which was contemplated as a partial or complete replacement for ration biscuits. The yeasts were assumed to be treated to overcome their gluten softening property. The 3 percent level produced a bread inferior in color, texture, loaf volume and flavor to the 1 percent level, rating bread scores of fair to poor as compared to very good and excellent for the 1 percent and control breads. Yeasty flavors and after tastes were detected in the 3 percent loaves. On storage these effects became more pronounced, especially the bitter, yeasty flavor and collapse of bread structure. In the 1 percent level breads yeast was not detected initially or on storage. The loaves, however, did not all have similar scores, varying somewhat in color, grain and loaf volume depending on the particular yeast.

The feasibility of adding yeast to the flour at the point of production rather than the point of baking was also investigated. Yeast was added to flour at a 3 percent level and the mixture stored for one year at room temperature and 100° F. Breads made from this flour at monthly intervals were compared to breads made from flour and yeast stored

---

[4] Taste tests were conducted by Dr. F. W. Dove and the staff of the Food Acceptance Research Branch. The foods were rated on a scale ranging from 10 excellent to 1 repulsive. Ratings over 5.5 were considered acceptable, those under 5.5 unacceptable.

[5] These yeasts were fortified prior to drying with synthetic thiamine, riboflavin, and niacin to contain 600, 600, and 1000 mcg. of these vitamins respectively per gram of dried yeast. This was done in order that the daily ration of biscuits would supply the difference between the thiamine, riboflavin, and niacin content of the ration and the amounts of these vitamins recommended by the National Research Council.

separately and from stored flour alone. The initial breads containing the yeast gave a total score of poor, rating lower in color, flavor and grain than did the breads made with flour alone. On storage, the bread scores of both the control and enriched flours decreased, the presence of yeast in the stored flour not delaying or hastening the deterioration.

In premixed cereal which is a ready-to-eat cereal containing dried milk and sugar the addition of primary grown and debittered brewers' yeast at a 1½ percent level could be detected especially when hot water rather than cold was used with it. Yeast in a diamalt mixture, however, imparted a very desirable flavor to the cereal when 12 percent of the weight of the cereal was used. On storage both the enriched and control samples declined in palatability but remained in the acceptable range through six months storage at 100° F.

In macaroni and noodles, additions of 3 to 15 percent yeast showed an increase in color of the finished product and a decrease in palatability as the yeast level was increased. Noodles fortified at a 3 percent level with both primary grown and debittered brewers' yeasts were served as boiled noodles, with braised beef and gravy and in a chicken noodle soup. The dishes containing the fortified noodles with the exception of boiled noodles were rated as acceptable as the control samples. On storage, the enriched noodles declined in palatability, but at the end of a year the products were still rated acceptable.

The addition of yeast to dehydrated soups was found not only to increase the vitamin content but also to enhance the flavor. Levels of 1 to 20 percent of approximately 20 different yeasts were used adding them to green pea, yellow pea and navy bean soup. The maximum thresholds varied from 1 to 10 percent for the different yeasts although 3 to 5 percent was satisfactory for most of the samples tested. On storage, for one year, both fortified and control samples deteriorated to the unacceptable point, bitterness and after taste being commented upon especially at the higher storage temperature.

In bouillon containing an 18 percent yeast-vegetable extract bitter and yeasty flavors along with increased pigmentation were noted in the beverages made from the stored samples. However, these were still considered drinkable by the testers.

The addition of a 3 percent level of a primary grown yeast to processed cheese could not be detected by the panel but on storage the color of the enriched cheese darkened considerably as compared to the control, and bitter yeasty flavors could be discerned by the panel. This level was therefore considered unsatisfactory for cheese enrichment.

The addition of yeast to chocolate in the form of U. S. Army Field Ration D Bar was made with several yeasts so that 2 mg. of thiamine per 2 ounce bar would be supplied. This proved quite satisfactory when dried primary or brewers' yeast was employed but unsatisfactory when a yeast extract was used. In hard candy the flavor of yeast was very effectively masked by the acidity and fruit or other flavoring. On storage the yeast flavor remained masked because of the terpene flavor and odor which developed.

The stability of the thiamine, riboflavin, and niacin contributed by the yeast was tested on storage as stated previously. Riboflavin and niacin were retained 90 to 100 percent in all products on storage for 6 months to one year at 100° F. Thiamine was retained to the same extent in foods of low moisture content such as chocolate, hard candy, dehydrated soups, noodles and macaroni, flour, premixed cereal, and ration biscuits. The retention of thiamine following 6 months' storage at 100° F. in foods of relatively high moisture content was 50 percent for canned bread, 25 percent for processed cheese, 60 percent for condensed chicken noodle soup, 55 percent for Tastyeast candy bar. Similar stability is obtained when these same foods are fortified with synthetic vitamins. Cooking losses sustained as a result of boiling the yeast-containing noodles were determined. It was found that 50, 50, and 65 percent of thiamine, riboflavin, and niacin respectively was retained in the boiled noodles, the remaining nutrients being almost completely recovered in the discarded cooking liquid. However, cooking the noodles directly in the soup gave complete retention of the three nutrients. In baking bread approximately 10-20 percent loss of thiamine was incurred but no loss of riboflavin and niacin.

Summary

Dried yeast, primary grown, and debittered brewers' may be incorporated into a large variety of foods without impairing acceptability. There was great variability noted among yeasts in the flavor, odor, color and texture characteristics imparted to the enriched food. The maximum threshold for yeast addition was determined not only by the type of yeast but also by the type of food, highly spiced foods taking higher levels of yeast enrichment than bland foods. Modification of basic recipes such as decrease in salt content and thickening agents were also essential to making yeast enrichment palatable. In bread and baked products the level of yeast was limited by the degree of gluten softening action of the

particular yeast unless the yeast was treated to overcome this effect. In most instances the 1 percent level of yeast addition could not be detected, whereas the 3 percent level which could not be detected initially in some foods developed the yeasty bitter taste on storage. It is therefore possible to add higher levels of yeast to foods to be consumed shortly after preparation than to those stored for long periods under unfavorable conditions.

Retention of the thiamine, riboflavin, and niacin of several yeast-enriched foods stored for 6 months at 100° F. was from 90 to 100 percent in the low-moisture foods. In relatively high-moisture foods riboflavin and niacin stability remained the same but thiamine was reduced from 50 to 75 percent depending on the food.

REFERENCES

1. Pavcek, P. Yeast and Nutrition Targets in Germany. FIAT Final Report. No. 195, Oct. 1945.
2. Prouty, C. C. Bull. No. 484, Jan. 1947. State College of Washington.
3. Skoog, F. Food Yeast, Production and Utilization in Germany. Tech. Int. Br. OQMG (1945).
4. von Loesecke, H. W. *J. Am. Dietet. Assoc.* **22**, 485 (1946).
5. Unpublished material. QM Food and Container Institute. Captured Rations.
6. Gortner, R. A., and Gunderson, F. L. *Chem. Eng. News* **22**, 160 (1943).
7. Mitchell, H. S. *J. Am. Dietet. Assoc.* **19**, 820 (1943).
8. Sure, B. *J. Am. Dietet. Assoc.* **22**, 114 (1946).
9. Sydenstricker, V. P. *Nutrition Abstracts Rev.* **12**, 339 (1943).
10. Thaysen, A. C. *Nature* **151**, 406 (1943).
11. Isker, R. A. *Brewers' Digest.* Aug. 1943.
12. Frey, C. N., Schultz, A. S., and Atkin, L. Proc. First Food Conf. Inst. Tech. **1**, 275 (1940).
13. McCollum, E. V. *Science* **102**, 181 (1945).
14. McCollum, E. V. *Maryland Health Bull.* **17**, 2 (1945).
15. Schwarz, R., Laufer, S., Laufer, L., and Brenner, M. W. *Ind. Eng. Chem.* **34**, 480 (1942).
16. Davis, C. F., and Frenkel, G. *Cereal Chem.* **14**, 100 (1947).
17. Carr, R. E. *Mich. State Bull.* 178 (1941).
18. Sure, B. *J. Am. Dietet. Assoc.* **22**, 494 (1946).
19. Durfee, S. T. *Brewers' Digest* **18**, 127 (1943).
20. Heller, C. A., McCay, C. M., and Lyon, C. B. *J. Nutrition* **26**, 385 (1943).
21. Neidert, M. Recipes with Brewers' Yeast. Dept. of Inst. Mgt. Cornell University (1943).
22. Neidert, M. Quantity Recipes Using Brewers' Yeast. Ibid.
23. Sunderman, R. C., Kahn, R. M., and Schmitt, H. T. *J. Am. Dietet. Assoc.* **21**, 81 (1945).
24. Sure, B. *J. Am. Dietet. Assoc.* **23**, 113 (1947).

25. Paul, P., Frueh, M., and Ohlson, M. A. *J. Am. Dietet. Assoc.* **24**, 673 (1948).
26. Cederquist, D. C., Bennett, B. V., Plummer, J. A., and Ohlson, M. A. *J. Am. Dietet. Assoc.* **24**, 676 (1948).
27. Roth, C., and Zander, E. *Medical Record* **157**, 741 (1944).
28. Davis, A. Let's Cook It Right. Harcourt Brace and Co. (1948).
29. Spinella, J. R. *J. Am. Dietet. Assoc.* **21**, 84 (1945).
30. Spies, T. D., Grant, R. M., and Grant, J. M. *Southern Med. J.* **34**, 159 (1941).
31. Personal Communication, Anheuser Busch.
32. Progress in Candy Research. Report No. 14. Cooperative project of Agricultural Research Administration and Nat. Conf. Assoc. April 1-June 30, 1948.
33. Brenner, S., Dunlop, S. G., and Wodicka, V. O. *Cereal Chem.* **25**, 367 (1948).
34. Brenner, S., Wodicka, V. O., and Dunlop, S. G. *J. Am. Dietet. Assoc.* **25**, 409 (1949).
35. Bollman, M. Unpublished material. QM Food and Container Institute.
36. Dunlop, S. G. *Modern Brewery Age* **33**, 24 (1945).

Experience in Use of Yeast in Community and Industrial Feeding

CLIVE M. McCAY
Cornell University, Ithaca, N. Y.

The author's interest in yeast was aroused during the period of 1921-23 in the laboratory of V. E. Nelson at Iowa State College, when Nelson, Fulmer, and Cessna were attempting to determine if yeast could synthesize water-soluble vitamins.

Because of the superior position of yeast in satisfying the nutritive requirements of species of animals ranging from insects to man, interest in yeast on the part of the author's group as well as other groups was continued. This is evident from the extensive literature which has been published.

Yeast In Animal Nutrition

Early work at Cornell demonstrated that yeast provided a unique supplement for such diverse species as cockroaches, clothes moths, bean weevils, and wheat weevils. Since few water-soluble vitamins were available in pure form at that time, the yeast was used as a source of B-complex vitamins. Possibly today these insects can be maintained upon synthetic vitamins. In most cases, this has not been tried and in all likelihood yeast may still contain undiscovered essentials necessary for the optimum nutrition of insects.

In addition to studying the part played by yeast in the diet of insects parallel studies were run for sixteen years upon brook trout. While yeast supplied part of the water-soluble vitamins for this lower vertebrate, it failed to satisfy all the requirements for this species. There are still unknown factors in raw meat that are essential for trout. Trout as yet have not been successfully reared and carried through reproduction upon synthetic diets supplemented with all known vitamins. The factors in raw meat are very thermolabile and may resemble those required by cats and mink. Apparently, both of these species require factors found in raw meat or milk but not available from yeast.

The value of yeast was also demonstrated in work with calves. Sickly, emaciated calves in which no parasites or diseases could be diagnosed, responded favorably when suspensions of yeast in warm water

were put into their rumens by tube. Within a few days the calves showed improved appetite and drank the yeast suspensions. Within a few weeks they were normal and had developed an appetite for the usual foods. The yeast had probably supplied some essentials for stimulation of the bacterial flora of the rumen. As far as is known today, synthesis in the rumen can provide all water-soluble vitamins and all essential amino acids. Conditions must arise at times when this synthetic power is partially lost due to an accident to some link in the chain of synthesis.

A number of studies have been made feeding diets containing up to 10 percent of dried yeast to dogs. Even growing puppies tolerate this high level. At the present time a group of four Great Dane puppies are being fed a diet containing no meat or milk but having 10 percent dry yeast in the mixture. These studies have only been in progress for two months and the results are as yet not known.

Most good dry dog feeds contain one to three percent of dried yeast. The flavor of yeast apparently is not readily acceptable to the dog. However, the author feels from past experience that flavors of yeast can be developed that will be palatable to the dog. This was demonstrated when some animal grade yeast that had probably been acted upon to a slight degree by some putrefactive organism before the yeast was dried, was readily consumed by dogs. This is not surprising as dogs usually bury bones in the soil prior to consumption; also many dogs have a marked appetite for horse or cow manure.

In the future, man may discover means of growing specific microorganisms upon yeast and thereby modify both its flavor and its content of some specific factors, such as vitamin B_{12}. Although most primitive people, such as Eskimos, make use of food products that have been changed through microorganisms, we are probably only at the beginning of an era of such processing. During the late war some of the white prisoners in Java were fed both soy bean meal and cocoanut meal after these products had been rendered edible through the action of a mold long used for such purposes by the primitive peoples of Java.

Extensive use of yeast has been made in studies in the field of gerontology. Rats have been employed throughout these attempts to determine the interrelationship between diet and aging. In the first studies in which the extreme span of life of the rat was greatly extended, all rats were fed a diet throughout life containing 5 percent of dried yeast. The oldest rats in this study exceeded 1400 days of age, whereas the average rat lives about 700 days. These data indicate that a substantial

level of yeast can be fed during the whole span of life without evidence of injury. This answers one of the common queries concerning the possible injury from the purines found in yeast. As far as we are aware there is no evidence of injury from any constituent of yeast.

Yeast In Human Nutrition

For centuries men have consumed yeast. Even two hundred years ago dried yeast was prepared by drying on thin paddles in the air. This yeast was used aboard ships for baking, although fresh bread was not used extensively in the Navy until about 70 years ago. In the old days men must have drunk substantial amounts of yeast in their beer. When the beer allowance was a gallon per man per day, each man must have drunk the equivalent of one to two teaspoons of dried yeast, if we assume that a spoon holds 3 grams and that a pound of dried yeast originates from 100 gallons of beer.

However, dried yeast as we know it today represents a new food. Past human experience with the introduction of common foodstuffs into the human dietary indicates that one or more centuries are usually required. Although the potato was introduced into Europe as early as 1588, it was not widely cultivated in Germany until two centuries ago. During the period of 1784-1795, Count Rumford was still struggling with the problem of inducing the Bavarians to grow and eat potatoes. We need not be discouraged if such valuable new foods as yeast and soy beans require one or two centuries for acceptance by an overfed American public.

Our own attempts to introduce yeast into our local community of Ithaca started about 1942. For some years before this, our laboratory was bothered by requests from people who desired to employ yeast as food. After considerable resistance the manager of the cooperative store was induced to retail dry, powdered yeast in pound and half-pound sacks. At the same time, instructions were issued concerning methods of incorporating such yeast in cooking and baking. Since that date the store has retailed yeast successfully although no advertising is devoted to it.

During the period 1942-43, a number of attempts were made to develop palatable tablets by combining yeast with dry skim milk. This proved to be a satisfactory combination and should be useful nutritionally if marketed more widely. Several attempts were also made to put yeast through a commercial pelleting machine. At first, the yeast was badly

scorched because it stuck when moistened with steam. However, good pellets were finally made by first mixing the yeast with alcohol. The production of pellets is much less expensive than the manufacture of tablets. Unfortunately, these pellets were not used even for the wartime export of yeast to Russia from the Boston area. The Russian yeast was shipped in the form of tablets. Germany, Japan, and Russia must have made substantial use of yeast, judging from its appearance in captured rations.

Our first test of the introduction of yeast into large-scale cookery was made at the Brooklyn Navy Yard in 1942-43. In this venture analyses and assays were first made to determine the adequacy of the meals served. Estimates were made of the two meals commonly eaten at home by the workers. The overall picture indicated that the diet might be improved substantially by the introduction of dried yeast into the noonday meal.

Many difficulties were encountered because the food service was in the hands of an inexperienced contractor who was not using standardized recipes. However, recipes were finally evolved and substantial amounts of yeast were introduced, especially into dishes with stronger flavors, such as meat. No objections were made by those consuming the dishes supplemented with yeast. However, the project was abandoned when one of the workers entered the Navy in 1943.

Testing of recipes continued in the Home Economics Department at Cornell, however, and mimeographed copies of both large and small scale recipes were issued.

One of the best foods for the incorporation of dried yeast has proved to be doughnuts. These are very satisfactory even when 10 percent of the flour is replaced with the dried yeast. Molasses cookies and similar baked products are also very satisfactory carriers of this product. A popular way of using yeast is the old method of mixing it with molasses for use on hot cakes and waffles. Another very satisfactory combination is in the form of ice cream sundaes in which the yeast replaces the nuts. Ice cream with small lumps of yeast incorporated in the mixture at the time of freezing might prove very satisfactory.

Two large channels of use would seem to be needed today in the improvement of human nutrition. For some years we have given special attention to the development of bread of higher nutritive value. During the past year the New York Department of Mental Hygiene which feeds more than 90,000 patients in twenty-seven hospitals has established a

new bread formula. This bread is made with 6 percent high fat soya flour and 8 percent dry milk solids.

In tests run in our laboratory none of the breads sold commercially in our community, with one exception, would support the growth of rats even when supplemented with 10 percent of butter, but this bread from the mental hospital did support moderate growth. The exception in our city was a bread of a formula similar to that from the mental hospitals but superior, inasmuch as this bread was made from an unbleached flour containing 2 percent wheat germ.

All of these inferior local breads, however, gave excellent results as indicated by the growth rate of the rats if the ground bread, mixed with 10 percent of its weight of butter, was further supplemented with yeast at a level of 10 percent of the mixture. Lower levels of yeast will be tested at later dates. No one knows how much the health of a community might be improved if the breads were made from a formula that contained 5 to 10 percent of dried yeast.

It is realized fully that the production of bread is a very competitive industry. Furthermore, there is probably no foodstuff that suffers more from the pinchpenny policies of the housewives than does bread. This is partly due to the fact that the housewife has no means of judging the nutritive value of bread.

For the latter reason and also in the interests of those who suffer from allergies to special products, it would seem desirable to print on the label of each loaf of bread a list of all special ingredients, such as yeast, soy flour, wheat germ, and dry milk solids. If this list also gave the amounts in percentages, the nutrition teacher would have some means for teaching the housewife to select breads of higher nutritive value. Such a program might revitalize the entire baking and milling industry. It might help restore the confidence of the housewife, the physician, and the nutritionist in bread.

Finally, brief consideration should be given to the problem of providing the children and adults of America with better beverages. Our figures which are over a decade old indicate that even ten years ago the soft drinks sold in the nation amounted to about two-thirds of a billion dollars. If we are to have healthy adults the country must have well-fed children. Today, the common soft drinks are composed of such ingredients as sugar, caffeine, phosphoric acid, organic acids, flavors, and coloring matter. It would seem that an enlightened technology could find means of working yeast back into acceptable beverages so that our nation would not have to let its children drink such mixtures as that listed above.

Utilization of Yeast in Chicken and Turkey Diets

JAMES McGINNIS
State College of Washington, Pullman

Use of dried yeast to supplement chicken and turkey diets dates from many, many years past. Under most conditions, yeast has been included because of its high content of B-complex vitamins. This role of yeast in poultry feeds is perhaps becoming less important with the increased production of the B-complex vitamins. Because of the high protein content of yeast, however, and the fact that yeast converts inorganic nitrogen and carbohydrate to protein, increased attention is being paid to the possible use of yeast as a commercial protein supplement in poultry feeds.

On the basis of both published and unpublished data, the protein in dried yeasts of various sorts can be utilized satisfactorily by poultry under some conditions. The number of experiments in which the protein value of yeast has been studied is relatively small when compared to the tremendous amount of work which has been conducted on its vitamin values. Temperton and Dudley *(1)* reported that laying hens utilized the protein in either a sample of *Torula* yeast or dried brewers' yeast for egg production, body weight, and maintenance of health as efficiently as they used the protein in fish meal. No harmful effects were noted when the diet of the hens contained as much as 12 percent of either *Torula* or brewers' yeast. The nutritional value of yeast protein for poultry did not measure up to fish meal when young growing chicks instead of mature hens were used as the experimental animals. Klose and Fevold *(2)* found that the protein in both *Torula* and brewers' yeast was significantly lower in biological value than fish meal or soybean oil meal. A combination of *Torula* yeast and soybean oil meal gave somewhat better results than the yeast alone. These workers also showed that a chick diet in which *Torula* yeast supplied all of the protein was deficient in both cystine and methionine. A marked increase in growth resulted when the diet was supplemented with 0.5 percent of methionine. It should be pointed out in connection with this work that the chicks used in both experiments were fed a practical type of starting diet until they were ten days of age. In terms of many experiments, the duration of the above trials in which the nutritional value of yeast was investigated, was rela-

tively short. In view of chick growth data which were obtained at Washington State College *(3)* in a trial in which the chicks were given the experimental diets at hatching time, it appears that diets containing high levels of yeast are better tolerated by chicks when given at an older age. A supplement of methionine to the diets containing *Torula* yeast grown on pear waste or *Torula* yeast grown on sulfite-waste liquor gave a growth response. In the case of the latter yeast the response was not nearly as marked. Extremely high mortality was encountered in this experiment. In both cases mortality in the groups receiving the yeast with a methionine supplement was higher than in the corresponding control groups. An explanation for this effect is not readily apparent. In addition to the differences observed in growth rate, one other important observation was made. Toward the end of the four-week feeding trial, the accumulation of large amounts of fluid under the skin and in the body cavity was noted in the chicks receiving the methionine supplement. A lower incidence of this condition was observed in the control groups. A gross examination showed extensive kidney damage.

In a subsequent chick experiment *Torula* yeast grown on different substrates was used to supply a part or all of the supplementary protein in a practical type of diet. The results obtained were markedly different from those mentioned above. In this experiment, the chicks were fed a regular starting diet until they were three weeks of age. At that time, they were distributed into the experimental groups and given the various diets. There did not appear to be any marked or significant difference between the growth which was obtained on the positive control diet in which soybean oil meal furnished supplementary protein and the other experimental diets in which yeast or combinations of yeast and soybean oil meal were used. Only one chick died throughout the course of this experiment. It appears then that yeast protein is much more satisfactory for chicks beyond one week of age, particularly when used in a practical diet in combination with other protein-bearing materials.

Experimental Work With Turkeys

In a series of experiments with turkey poults, dried brewers' yeast and *Torula* yeast grown on pear waste were used as a supplement to a practical type of starting diet deficient in riboflavin. The yeast was added to this diet at the expense of an equal amount of soybean oil meal since analyses showed these two ingredients to be similar in protein content.

In the first experiment, dried brewers' yeast was added at graded levels up to 10 percent of the diet. We were interested in determining

the effect of the dried yeast on the incidence of dermatitis, the incidence of perosis, and on rate of growth. It should perhaps be pointed out that the principal effect of the yeast might be due to its addition of riboflavin since the diet was deficient in this vitamin. From the results obtained in this experiment, it is evident that the 2 percent level of yeast gave a pronounced increase in growth and markedly reduced the incidence of both dermatitis and perosis. Growth was not improved by the addition of yeast above the 2 percent level. However, the dermatitis and perosis were prevented by levels above 4 percent.

In the next experiment with turkey poults, the effectiveness of brewers' yeast was compared with *Torula* yeast grown on pear waste. In this experiment, the yeast did not prevent the perosis as effectively as in the previous experiment. The type of perosis observed in the groups receiving yeast was not typical of that observed on the basal diet in that it was much milder in form. The results obtained in the group receiving only the supplement of crystalline riboflavin are of interest since growth was only slightly lower than that obtained with any level of yeast. There was, however, a somewhat higher incidence of perosis than that observed in many of the yeast-fed groups. A combination of dehydrated alfalfa and a fermentation riboflavin concentrate gave the same growth and prevented both dermatitis and perosis. On the basis of these experiments with turkey poults, it appears that either brewers' or *Torula* yeast grown on pear waste may be depended on as a riboflavin supplement and as a protein concentrate to the extent of 10 percent in the starting diet.

Experiments With Laying and Breeding Hens

Experiments have been conducted with laying and breeding hens in which *Torula* yeast and brewers' yeast were used to supply a part or all of the supplementary protein. In these experiments New Hampshire hens were kept in laying houses with a litter floor. It was of particular interest to determine whether or not a high level of either *Torula* or brewers' yeast would supply an unidentified factor which is required for hatchability in chickens. Previous work had shown that lower levels of brewers' yeast did not increase hatchability when used as a supplement to a diet containing soybean oil meal.

Approximately 2 percent of fish meal was used as a supplement to the *Torula* yeast and the soybean oil meal.

Each of the experimental diets was fed to duplicate groups of approximately 25 hens each.

Satisfactory egg production was obtained with all of the different diets. Because of the variation in the rate of egg production between duplicate groups, no significance should be attached to any differences which were observed in this experiment. The hatchability results were surprising since lowered hatchability was anticipated especially with the brewers' yeast. Earlier work had shown that this material could be fed to breeding hens at a level of 5 percent, without improving hatchability or to chicks without improving growth. The results on yeast, where no fish meal was fed, was expected to be similar to the results obtained with the soybean oil meal. However, hatchability was satisfactory for all diets except the one containing soybean oil meal.

In order to obtain further information confirming or disproving the presence of the unidentified factor within the yeasts in any significant quantities, chicks hatched from hens fed each of the experimental diets were wing banded, divided into two groups and placed in battery brooders. One group of chicks was fed a chick diet known to be deficient in a factor required for chick growth, whereas, the second group was fed this same diet with a supplement of fish meal. Previous work had shown the factor required for hatchability to be deposited in the egg in sufficient quantities to provide a carry-over for chick growth. In contrast, when chicks hatched from breeders fed a diet low in this factor were fed a deficient starter diet, growth was extremely poor and mortality high. The results obtained with chicks in this trial clearly indicated a significant carry-over of an unidentified factor from the breeders. Two explanations are possible. Either the samples of yeast contained the unidentified factor required for hatchability and for chick growth or the presence of yeast in the diet caused the microbial synthesis of this factor in the intestinal tract or in the feces after voiding. Considerable work has shown that hens may obtain significant quantities of the unidentified factor by consumption of feces. For more direct evidence on either the presence or absence of the factor in *Torula* yeast, two different samples of this material were fed to chicks. One sample of the yeast was grown on a semi-synthetic medium, whereas, the other sample was grown on a medium in which pear juice supplied the carbohydrate. The chick growth results in this experiment were entirely negative since neither sample of *Torula* yeast gave a growth response over the basal diet. This would indicate then that the yeast samples together with some other environmental factor perhaps caused a synthesis of the factor in the droppings of the hens.

A second experiment with hens was conducted in which *Torula* yeast grown on citrus waste was added to the diet at graded levels. The experimental set-up and conditions for this experiment were similar to those of the previous experiment. There was, however, one difference which may have been of importance in explaining the results. A smaller number of hens was used in each of the experimental lots which would decrease the amount of droppings in the litter. In addition, the litter was changed in the pens during most of the experiment at weekly intervals. In the previous experiment, because of labor shortages and so forth, the cleaning was less frequent.

Again, each of the experimental diets was fed to duplicate groups of hens. The results on egg production were satisfactory. As was observed in the previous experiment, no differences in egg production occurred. The yeast was used as efficiently as soybean oil meal for this purpose.

In contrast to the hatchability results which were obtained in the previous experiment, the yeast supplements did not improve hatchability or at least not very markedly. A small supplement of fish meal added to the soybean oil meal again gave an increase in hatchability of fertile eggs.

When chicks hatched from hens fed the different experimental diets were placed on a chick diet deficient in a factor required for growth, extremely heavy mortality occurred in all chicks except those hatched from the breeder diet which contained a small amount of fish meal.

Summary

In summarizing, it appears that yeast of different types may be used satisfactorily in chick diets providing it is not fed at too high a level or at too early a period in the chick's life.

The work with turkey poults showed that either *Torula* or brewers' yeast could be used as a substitute for up to 10 percent of soybean oil meal. In addition, it was an effective riboflavin supplement for turkeys.

The experiments with laying hens in which *Torula* yeast was used as a protein supplement showed it to be satisfactory for egg production but not for hatchability. Yeast in breeding rations should be supplemented with some material such as fish meal which contains an unidentified factor required for reproduction, probably vitamin B_{12}.

REFERENCES

1. Temperton, H., and Dudley, F. J. *Harper Adams Utility Poultry J.* 26, 172 (1941).
2. Klose, A. A., and Fevold, H. L. *J. Nutrition* 29, 421 (1945).
3. McGinnis, J. Unpublished data.

Utilization of Yeast in Livestock and Specialty Feeds

R. V. BOUCHER
Department of Agricultural and Biological Chemistry,
The Pennsylvania State College

Success in the livestock industry is dependent upon high rates of production. As greater productivity has been bred into all classes of livestock the efficiency of feed utilization has increased. This is reflected in more rapid gains in growing animals and in higher rates of production in adults per pound of feed consumed, whether it is in pounds of milk or dozens of eggs.

A generation ago the longhorn cow, the razor-back hog and the spotted hen survived very well on what they could forage for themselves. Today, however, in order to maintain the high rates of production of which modern highly bred animals are capable, qualitatively complete and abundant nutrition is absolutely essential.

That brings us to the subject of how yeast fits into modern rations for livestock, pets and furbearers. The best summaries of generally accepted facts regarding nutritive requirements of swine, dairy cattle, beef cattle and sheep are found in the Recommended Nutrient Allowance Reports of the Committee on Animal Nutrition of the National Research Council. Information on the requirements of other animals including horses, dogs, cats, goats and furbearers must be obtained from the original literature and in some instances the data are found to be far from complete. Conspicuous by its absence is information on the requirements for many of the vitamins of the B-complex. Yeast plays an important role in modern rations by supplying many of these essential nutrients.

Swine

High mortality in young pigs is the most serious problem of the swine industry. Inadequate nutrition during the period of gestation and lactation is generally accepted as the chief cause of these high losses. Research at the agricultural experiment stations indicates that ordinary farm rations fed to sows in dry lot during pregnancy and nursing periods are likely to be most deficient in vitamins of the B-complex. Even carefully formulated sow and pig rations, known to contain adequate amounts

of protein, carbohydrate, fat, salt, calcium, phosphorus, manganese, iodine and vitamins A and D, as well as appreciable amounts of niacin, pantothenic acid and riboflavin failed to support the pigs properly, until the rations were supplemented with good natural carriers of the vitamin B-complex. Among the best and most economical carriers of these essential vitamins for swine are brewers' dried yeast, fish solubles and alfalfa meal. Much remains to be done in determining the quantitative requirements of the known vitamins but the practical solution of the problem need not wait upon completion of that work as long as adequate quantities of the natural carriers of the vitamin B-complex are available.

Cattle

Few reports occur in the literature on experiments with yeast in rations for either dairy or beef cattle. Considerable work has been done, however, with yeast in dry calf starter diets. Laboratory experiments have not given clear cut results, but the practical experience of many feed manufacturers is so favorable that for years they have been using 2 or 3 percent of brewers' dried yeast in their calf starter formulas.

Dogs

The nutritionally critical period in the life cycle of dogs is during reproduction and early growth, just as in all other animals. It is relatively easy to supply the nutritional needs of an adult male or non-pregnant female but during gestation and lactation there is a rapid rise in the requirements for many of the essential nutrients.

Proteins, fats and minerals are easily supplied as are the fat-soluble vitamins. The requirements for the water-soluble vitamins of the B-complex are less completely understood and appear to be relatively high during the reproductive period. Brewers' dried yeast is particularly effective at this period in supplying thiamine, riboflavin, niacin, pyridoxine, biotin, folic acid, choline, pantothenic acid and the other less well-known as well as some of the as-yet-unidentified members of the vitamin B-complex.

The practical applications of our knowledge of dog nutrition may be summarized in the words of Michaud and Elvehjem *(1)*, "If the diet is made up largely of cereals, several additions must be made to render it complete. Sources of protein, riboflavin, nicotinic acid and calcium must be added. The protein may be added as meat or meat scrap, the calcium as bone meal or calcium salts, and yeast or liver may be used for the B vitamins."

Furbearers

Much less is known about the specific nutritive requirements of furbearers but in general their requirements are similar in most respects to those of dogs. In order to provide for the relatively high needs of mink and fox for vitamins of the B-complex, brewers' dried yeast has been widely used for years at levels of from 2 to 5 percent of the dry portion of the ration. Yeast seems to be particularly effective in preventing skin disorders and in helping to assure pelts of highest quality.

Cats

In view of the extensive work that has been done on dog nutrition, it is surprising to find not more than a dozen papers in the scientific literature dealing with the nutritional requirements of the cat.

Heath in 1940 reported the production of pellegra in cats *(2)*. The symptoms disappeared when 30 to 80 mg. of niacin were administered daily. Yeast would probably have been equally effective or perhaps even more effective as it is when administered to dogs suffering with black tongue.

Fish

Investigations in the feeding of fish have dealt primarily with carp and trout. Most of the reports on carp are found in the older German literature. Trout have been extensively studied in the United States because the feed bill of the nation's fish hatcheries even before the war exceeded a million dollars per year. The objectives of trout research have been to reduce this cost and to produce healthier fish for release in streams.

Most trout hatcheries whether they are operated by the government or private concerns are interested primarily in producing fish for stocking streams. A few large hatcheries in states like Colorado produce trout on a substantial scale for sale as food. In foreign countries trout are likewise reared primarily for sport fishing.

From the beginning of the rearing of trout in hatcheries about a century ago, extensive use has been made of meats, usually offal such as liver or horse meat. Since research upon the nutrition of trout was started about 1927, extensive substitutions have been made for meat by the introduction of various dried products such as dry milk solids, cotton seed meal, wheat flour and yeast.

During the feeding of canned fish to trout in Wisconsin hatcheries it was found necessary, as reported by Schneberger *(3)*, to add brewers' yeast to the diet in order to prevent thiamine deficiency.

Some factors in yeast seem to protect trout against Gill disease according to the findings of Wolf *(4)*. Pantothenic acid seems to be one of the factors provided although other water-soluble vitamins are also involved. Earlier German work *(5)* had indicated the operculae (protective covering of the gills) of fish were shortened if the diet lacked yeast.

From the few nutrition experiments conducted with goldfish it seems that B-complex vitamins are important in helping to protect the goldfish against fungus infections *(6)*.

Honey Bees

Another specialty feed that contains brewers' dried yeast is a pollen substitute for honey bees. It contains 1 part of brewers' dried yeast and 6 parts of soybean flour. It is frequently fed dry but may be mixed with granulated sugar and water to form a candy for use in the apiary during the late winter season.

Summary

In summary it may be said that dry yeast (non-fermentive) is among the richest and most dependable sources of all the members of the vitamin B-complex. This characteristic accounts for its wide use in animal nutrition where it is particularly valuable during nutritionally critical periods such as gestation, lactation, and early growth.

REFERENCES

1. Michaud, L., and Elvehjem, C. A. *Nutrition Abstracts Rev.* 13, 323 (1944).
2. Heath, M. K., MacQueen, V. W., and Spies, T. D. *Science.* 92, 514 (1940).
3. *Progressive Fish Culturist.* 17 (Dec. 1941).
4. *Fisheries Research Bulletin No. 7,* New York State Conservation Department, Albany (1945).
5. *Biol. Generalis.* 8, 73 (1932).
6. *Trans. Am. Fish. Soc.* 63, 338 (1933).

OTHER PAPERS IN THE YEASTS IN FEEDING SERIES

Other phases of *Yeasts in Feeding* presented at the Symposium are given in this brochure in abstract or condensed form. After the Symposium was held many of these excellent papers were submitted to the journals or found other publication outlets. In cases where information as to the source of publication is not supplied by a footnote, it is suggested that those who have an interest in the original paper make application directly to the author as to where it may be obtained.

Relation of Basic Investigations to Production and Utilization of Yeast

M. A. JOSLYN
Division of Food Technology, University of California
Berkeley, California

Yeast has long been used as a favorite test object in the fields of general and comparative biochemistry and physiology, and much of our knowledge of carbohydrate, lipid, and vitamin metabolism has been obtained in investigations with yeast. *Saccharomyces cerevisiae* has been used more often in developing our knowledge of general physiology and biochemistry than any other species of yeast. A systematic investigation of the biochemistry of the well-recognized species of yeasts for the purpose of discovering natural relationships and extending our knowledge of the intermediary metabolism of the more important cellular constituents has not been undertaken.

Biological Syntheses By Yeasts

Fat Synthesis: The basic investigations on the mechanism of the conversion of carbohydrates to fat in yeasts have been most fruitful. The more important factors governing lipid production—oxygenation, carbohydrate and phosphate requirement—were established through yeast studies. It was also shown that the incorporation of acetic acid and ethyl alcohol in an aerated medium containing metabolizing yeast gives rise to an appreciable increase in fat content of the cells; this was attributed to the utilization of the added compound rather than the stored carbohydrates.

Protein Synthesis: Although the conditions favoring growth and protein production, particularly by *Torulopsis utilis*, are generally known, the chemistry of protein synthesis is still largely unknown. The recent

successful use of tracer technique in studies of protein synthesis in tissue slices and in tissue macerates may possibly be applied in investigations of the mechanism of protein synthesis by yeast. An examination of the amino acid composition of proteins of other species of yeast is highly desirable in order to obtain a source with the desired high-cystine and -methionine, and low-purine content.

Vitamin Synthesis: The synthesis of vitamins by yeast varies markedly with species, strain, and race, and with growing conditions. Growing conditions influence not only the quantity of a given vitamin but also the type of combination and nature of the particular isomer present. In recent years there has been great interest in the requirements of growth-promoting nutrilites for various yeasts, and many investigations have been carried out to determine the requirements for "vitamins" by yeast, not only for the purpose of completely defining an adequate medium, but also for the purpose of investigating the basic factors involved in vitamin synthesis. Some factors influencing the vitamin content of brewers' and bakers' strains of *S. cerevisiae* and of certain other food yeasts are composition of medium, degree of aeration, agitation, and cell yield.

Growth and Assimilation

The most fundamental approach to growth was due to Kluyver's concept that dissimilatory processes may well serve as a source of raw material for the assimilatory processes; that is, that anabolic reactions may occur spontaneously if the proper initial substances are provided by the catabolic activities of the cell. This concept has been useful in studies of growth and assimilation by *S. cerevisiae* as well as other microorganisms. These studies, in general, have shown that definite quantitative relationships exist between the amount of a foodstuff dissimilated and the amount assimilated by nonproliferating cells. With some organisms, synthesis during growth closely approaches assimilation observed in cell suspensions. Both oxidative and fermentative assimilation have been observed with yeast.

Role of Oxygen In Growth and Metabolism

Although aeration has been established as a growth requirement, little is known concerning the biochemical mechanism of aeration. The general belief is that oxygen is required for continuous growth of yeast and that the yeast can utilize only the oxygen dissolved in the liquid. Aeration is necessary to replace the dissolved oxygen, to remove fermentation products from the surface of the cells, and to substitute fresh nutrients. There are no essentially anaerobic organisms among species of yeast, but yeast may multiply in the absence of oxygen. Marked cytological differences and changes in synthetic ability occur with the change from aerobic to anaerobic culture. Although aeration stimulates the growth of yeast, the oxygen content of the gas used affects growth. The fact that pure oxygen is not so good a growth stimulant as air has been con-

firmed industrially. The quantity of air used, and the conditions under which it is introduced, distributed, and kept in the medium, are important factors influencing the yield. Agitation as well as aeration influences growth rate and yield, but this factor has received much less consideration.

A very striking feature of the yeasts is the range of respiratory and fermentative intensity which they exhibit and the marked differences in the Pasteur-Meyerhof effect among the various species of yeast investigated. It is well established that oxygen influences not only the nature of the products but also the rate of fermentation and substrate decomposition. It is possible that a more rational basis for oxygen requirement for growth could be obtained through elucidation of the mechanism of the Pasteur-Meyerhof effect.

Autolysis

The liberation of endocellular enzymes and proteins and the simultaneous enzymatic degradation by cellular enzymes of the cell substance, which constitute the process of autolysis, are phenomena useful in the preparation of yeast extracts although they are undesirable in the production of bakers' yeast and in the fermentation industries. Knowledge of the process of autolysis is necessary for both its control and utilization. The composition of the yeast (particularly its glycogen and protein content), pH and temperature are known to affect the rate and extent of autolysis, but the mechanism of the process remains to be studied.

Genetic Control of Yeast

When the qualities desired in a food yeast can be defined in terms of the biochemical reactions concerned, it should be possible to develop these characteristics by selection, adaptation or hybridization. The hypothesis that in all cases in which genes control specific reactions they do so indirectly through enzymes, has been well established. The genes concerned apparently determine whether or not specific enzymes are present in active form. Even though biochemical genetics is still in its infancy as a field of science, it has been most useful in elucidating the mechanism of several biochemical transformations. The phenomenon of enzymic adaptation has also been applied in biochemical investigations of intermediary metabolisms.

Basic Principles of Culturing and Production of Yeast

LAWRENCE ATKIN [1]
Wallerstein Laboratories, New York

Yeast for feeding may be obtained as a by-product or it may be the primary object of manufacture. Primary production, unlike by-product utilization, is capable of more or less indefinite expansion, and also affords flexibility in choice of culture, method of manufacture and raw materials.

Yeasts are cultured by standard bacteriological methods. Some 14 species of yeasts or yeast-like organisms have been cultivated for food use. These species fall within the following genera: *Saccharomyces, Zygosaccharomyces, Torulopsis (Torula), Monilia, Candida, Endomyces, Willia, Oidium, Fusaria,* and *Rhizopus.* As present yeast nomenclature is not entirely adequate, significantly different subspecies, varieties or strains may occur within a given species.

Choice of a strain for food yeast manufacture depends upon the end-product desired, the nature of the manufacturing process, and the substrate to be used, including the possible presence of toxic substances in the latter. Flavor has been a handicap to the wide use of food yeast; some unpleasant flavors may be inherent (as in undebittered brewers' yeast), or may result from the antifoam agent employed or the fact that yeast has been allowed to putrefy during processing. Flavor is less critical if yeast is used for animal feeding, but taste acceptability should be given consideration here also.

The nutritional value of yeast depends upon its protein and vitamin content. These characteristics may be influenced by strain used as well as by manufacturing method.

Other factors to be considered in choosing a culture are growth rate, yield, cell size, cell density, tendency to settle, optimum growth temperature, resistance to autolysis and resistance to involution. Rate of cell multiplication alone is not a criterion, as cell size and cell density must also be considered. Cell size will affect the ease of filtration and density will influence the ease of centrifugal separation. Cultures that tolerate high temperatures may be useful where a means of cooling during manufacture is not available. Resistance to autolysis is important because the cycle of yeast manufacture may extend over as long as three weeks.

When the proposed substrate is known to be inhibitory to yeast growth it is customary to "acclimatize" the culture to the substrate by means of gradually increasing concentrations of the new substrate in

[1] Present address—The Fleischmann Laboratories, 810 Grand Concourse, New York 51, N. Y.

the early stages of propagation. Some cultures are more readily acclimatized than others. If the proposed substrate is deficient in one or more of the bios factors required by the yeast it is usually possible to add a bios concentrate, or the difficulty may be overcome by selecting a less fastidious yeast culture. Some yeasts, e.g. *Torula utilis*, are able to utilize pentose sugars and thereby give higher yields than *Saccharomyces* yeasts.

Pure yeast cultures are maintained on agar slants, on agar slants covered with sterile oil, in 10% sugar solutions, in liquid malt wort solutions, or by lyophilization. The last method, although attractive, may permit mutation or segregation of characteristics and consequently should be used with caution.

Absolute sterility is not always necessary in the final yeast manufacturing stages. Adaptation of the culture to the substrate, oxygen tension and temperature of the commercial scale process should be accomplished gradually.

By-product brewers' yeast requires collection, debittering, and drying to produce a dried yeast product. Feasibility of collection depends upon economic factors. Reducing the number of fermentation cycles for which a given yeast batch is used in the brewery might increase the quantity of by-product yeast available but the desirability of this practice is not established. Primary production is necessarily the principal source of yeast for feeding since it is independent of beer consumption and may be expanded at will to accommodate the need for by-product disposal or any other requirement.

Cane or beet sugar molasses until lately has been the principal raw material for yeast manufacture. Wood sugars, produced either by direct acid hydrolysis or from the waste sulfite liquor of paper pulp manufacture, are coming into prominence. Agricultural wastes such as fruit and vegetable residues have also been used. Beside sugars, yeast substrates must provide bios factors, mineral salts and assimilable nitrogen. Adjustment of pH is customary, and sterilization of the substrate or wort before addition of the yeast is usually desirable.

The term assimilation is proposed as being a better one than fermentation to describe the commercial growing of yeast. Assimilation is based on the Hayduck principles of dilution, neutralization, and aeration. Dilution is maintained by gradual addition of nutrients during assimilation. Neutralization, to offset acidity resulting from yeast growth, is accomplished with aqua ammonia, which at the same time furnishes nitrogen. Urea can also be used as a nitrogen source, but requires additional biotin. The volume of air required for assimilation is large unless supplied as microbubbles. Fine dispersion of air may be accomplished by the use of ceramic spargers or by mechanical means.

Foam control during the aeration phase of propagation may be a problem, particularly with sulfite liquor. Foam is controlled by adding surface-active oily materials or by use of a mechanical device that draws the foam below the liquid surface.

Food yeast manufacture is usually a batch operation, although continuous processes are available that run uninterrupted for upwards of two weeks. At the end of assimilation, centrifugal separators are used to concentrate the yeast to about 15% of solids (half that of compressed yeast). Since it is not desired to maintain viability in the final product, feeding yeast may be dried at high temperatures. It is commonly dried in spray or drum driers. More knowledge is needed on the possible effects of high drying temperatures on nutritive values, i.e. possible protein damage. Flavor deterioration upon storage has been observed which may be due to rancidification of the antifoam agent used.

Aside from its use as a B vitamin source, feeding yeast is used principally as a source of dietary protein. The combination of microbiological protein production, as exemplified by yeast propagation, and sugar beet production will produce about three times as much food per unit acreage as soy bean cultivation.

Brewers' Yeast-Recovery and Preparation for Use

W. L. HEGARTY
M. K. Goetz Brewing Company
St. Joseph, Missouri

Brewers' yeast used in the fermentation of American beers and ales belongs to the species *Saccharomyces cerevisiae*. Brewers' yeast is predominantly derived from "bottom fermented" or "lager" beer. Beer yeasts are "fermented yeasts," grown at low temperatures under anaerobic condition. In this important respect they differ from top fermenting ale yeasts and primary grown yeasts, which are "respiration yeasts" cultured in aerated media at somewhat higher temperatures. This difference in culture conditions reflects itself in certain distinct characteristics and properties specific for each type of yeast.

Among the major differences between "fermentation" and "respiration" yeasts are: (1) the enzyme systems involved in the two types of fermentation, (2) the function and utilization by the yeast of certain components of the vitamin B complex, and (3) their capacity to ferment specific sugars.

Under equal conditions "respiration" yeasts are usually lower in thiamine and niacin, but somewhat higher in riboflavin than "fermentation" yeasts. Thiamine which is present in yeast as cocarboxylase and niacin which is part of the co-zymase system are both involved in fermentation, whereas riboflavin is part of the respiratory enzyme system. Yeasts high in riboflavin generally are also high in glutathione, and those

low in glutathione are also low in riboflavin, indicating a relationship between riboflavin and glutathione.

The two types of yeast also vary in their cytochrome spectra, respiration yeasts having a four band spectrum while the fermentation yeast spectrum has only two bands. The character of the cytochrome spectrum depends entirely upon aeration. The same yeast changes its spectrum when grown under reversed conditions. Another difference, presumably also due to the variation in enzymatic activity, is the behavior of the two yeasts when subjected to autolysis. The two also differ in the formation of ascospores—brewers' yeast having practically lost its capacity for spore formation. Differences in carbohydrate metabolism also are demonstrated by the proportion of gums formed: brewers' yeast, 4 to 6 percent; wine yeasts, 6 to 8 percent; bakers' yeast, 8 to 12 percent.

Since most other industrial and nutritional yeasts are grown under aerobic conditions, brewers' yeast is practically the only "fermentation" yeast and, as such, possesses some unique properties. Otherwise, yeasts display a great adaptability to the composition of the media in which they are grown and to other environmental conditions. A striking example of this property is the acclimatization of brewers' yeast to hopped beer wort.

The four- to six-fold increase of yeast cells during the fermentation from wort to beer, which results in a surplus of approximately one-half pound of dry yeast solids per barrel of beer, represents nutritionally the most valuable by-product of the brewing industry. When dried or otherwise processed (in form of extract, hydrolyzates, etc.) brewers' yeasts can make significant contributions to the American diet as a source of essential nutrients and flavor, in addition to furnishing a valuable feed stuff for poultry and livestock. Of the approximate total of 45,000,000 pounds of surplus brewers' yeast (on the dry basis) available annually, about one-third—twelve to fifteen million pounds—are recovered at present by drying in individual breweries or central drying plants, or by processing into various derivatives.

Brewers' yeast is one of the richest natural sources of the entire vitamin B-complex, as well as an excellent source of protein, minerals and other important food factors which are rendered readily assimilable by drying or extraction. The protein of brewers' yeast contains all essential amino acids and ranks, in biological value, between high quality animal proteins and the better plant proteins. Its composition, properties and nutritive value recommend the use of brewers' yeast as a desirable ingredient in many foods, as well as in feed formulas, especially as a supplement to cereals, refined cereal products and other foods high in carbohydrate content.

Special processing operations are required depending upon the purpose for which a specific brewers' yeast product is intended. These operations include debittering by washing with mild alkaline solutions, drying, smoking, etc.

Utilization of brewers' dried yeast can and does play an important part in animal and human food nutrition.

Dry, Extracts, Pastes, Hydrolysates, Autolysates, Flavors

J. E. McCLARY
Director of Research, Anheuser-Busch, Inc.
St. Louis, Mo.

While the food value of yeast or yeast extracts has been recognized for many years, little use was made of it until the discovery of the role vitamins play in nutrition, and the further discovery that yeast is one of the richest sources of water-soluble vitamins.

From the beginning of World War II, with the increased food shortages the world over, the use of yeast as a source of protein as well as a food supplement has increased and has been widely publicized. That yeast has not played a greater role in relieving the shortage of food, particularly protein, is due to at least three important factors: (1) the taste is not wholly acceptable to most people; (2) the cost of production is greater than that of other plentiful vegetable proteins; (3) the total yeast from brewing operations is relatively small.

Use

Food products from yeast so far have played only a minor role. The greatest use has been made of whole non-viable yeast and yeast extract as a source of B vitamins. Yeast was especially important as a vitamin supplement in the pharmaceutical field prior to the development of the synthetic vitamin industry. Since 1935 the most popular B vitamins—thiamine, riboflavin, and niacin—have been produced synthetically at such a low price that yeast and yeast derivatives find it difficult to compete. With an increasing recognition that the well known synthetic vitamins do not wholly answer the need for vitamin supplementation, the importance of rich natural sources of B vitamins has been increasingly recognized.

Sources of Yeast

There are three major sources of supply of yeast for food purposes: (1) Excess yeast *(Saccharomyces cerevisiae)* from the brewing and distilling industries, (2) primary grown yeast *(Saccharomyces cerevisiae)*, and (3) Torula type yeasts (usually *Torulopsis utilis*) primary grown on molasses or wood waste.

Non-viable Dried Yeast

For animal feeding surplus brewers' yeast, untreated, is dried on a conventional drum drier. For human consumption it is necessary to remove bitter substances from the surplus yeast. This is accomplished by an alkaline wash which has been modified only slightly since 1895. Surplus brewery yeast is sometimes regrown in a manner similar to

bakers' yeast production. The bitter substances are removed, and those remaining are diluted.

Yeast Extracts

One of the first commercial yeast extracts was prepared by a process patented by Wahl-Henius in 1894. The yeast was debittered by washing with an alkali, boiling for 30 minutes, and removing the insolubles. This extract was used as a medicinal preparation or tonic, though vitamins were not known then. Several patents have been granted for extracts based on minor variations of this process.

Hydrolysates

From a practical standpoint of yield and taste acceptability the use of acids, particularly weak acids, is most desirable. The methods are fully described in the patent literature.

In general, the acid or alkaline treatment leads to a greater recovery of the proteins and vitamins than is possible with aqueous extracts. Alkaline treatment is not desirable because the vitamins, particularly thiamine, would be destroyed. By acid treatment the protein is hydrolyzed to amino acids and peptones and the water-soluble vitamins are released from the complex organic compounds in which they are bound. Although treatment with heat and acid also destroys much of the unpleasant yeasty taste, the product is not palatable in large quantities.

Autolysates

The use of extract of autolyzed yeast has been given impetus since 1940, with the recognition that pre-digested proteins or mixtures of amino acids are useful in the treatment of certain diseases or abnormal physiological conditions.

For production of an autolysate any fresh commercial yeast or debittered brewers' yeast may be used. However, the use of yeast grown under controlled conditions will improve and standardize the quality of the product. Culture conditions have a marked effect on protein content, mineral content, and on the nutritional quality of the yeast.

Yeast autolyzes rapidly when it is placed in a tank in a concentration of about 15 percent dry solids in water, the temperature held at about 50°, and the slurry agitated. To speed up the process small amounts (1 to 3 percent) of a solvent such as ethyl acetate, or a salt such as sodium chloride, may be added. The yeast is held under these conditions for 12 to 24 hours, depending on the degree of breakdown desired.

Several methods have been described for separating and concentrating the solubilized yeast solids from the residues. A coagulant such as alcohol, followed by filtration, has been used. Concentrates in the form of syrup or paste may be produced by evaporation under reduced pressure, and dry products may be produced by spray or vacuum drying.

An autolysis of this type is probably the only method of producing an extract of yeast which retains essentially all of the nutrient value of yeast.

and in addition, there is some evidence that bound vitamins or conjugates are freed, which may lead to increased utilization.

SUMMARY

One of the most important objections to yeast is its taste. None of the yeast products are acceptable for making up an appreciable part of the diet, and their use has been restricted to food supplements to supply nutritional requirements, or to use in special flavors or meat substitutes where the amount consumed will be relatively small.

The taste of dry yeast in food products can be covered when the total amount included is relatively a small part of the formula. In many foods the proportion may be 10 to 20 percent of the total solids without materially affecting the taste. Efforts will doubtless continue to produce more palatable yeast products, either through treatment of existing yeast, or through the development of new species and strains. The taste of yeast of various strains differs when grown under similar conditions. However, all which have been studied at the Anheuser-Busch laboratories have been similar in taste. None of them is completely acceptable as a major item in the diet.

Once the problem of palatability has been overcome and the significance of yeast as a source of vitamins is generally understood we may see a much increased use of yeast and yeast products as a food.

Commercial Production Practices for Growing Primary Yeast for Nutritional Purposes[1]

CHARLES H. KEIPPER
Red Star Yeast and Products Company
Milwaukee, Wisconsin

It is a scientific endeavor to produce a primary grown yeast for nutritional purposes with specified qualifications. The actual production may be divided into (1) handling, clarification and sterilization of the best raw materials available; (2) selection of the culture and the subsequent propagation to produce a good stock yeast; (3) fermentation of the substrate with the stock yeast to obtain a yeast of required characteristics; (4) concentration, washing and drying of the finished yeast; and (5) milling, packaging and storing to ensure no loss of quality.

In the selection and treatment of the raw materials one must bear in mind that yeast cells require food in a soluble state. Present day conditions enable the yeast companies to use beet or cane molasses most advantageously.

[1] The original paper will appear in *Food Industries.*

The selected molasses are blended as desired and heated before clarification. Filters, separators or flocculation and sedimentation may be used to produce a clear solution. The wort holding tank is used as a sterilizer before the wort is pumped through coolers to the fermenters. Heat exchangers may be used if desired. At the time of starting the fermenters, chemicals, particularly ammonium salts, may be added and as the fermentation progresses further additions of both wort and chemicals are necessary. The fermenters may be of wood or metal and must be equipped with a large amount of cooling surface. The fermentation is greatly enhanced by agitation and/or aeration, in fact, it is not economical to grow yeast in the absence of one or the other. The usual practice is to introduce large quantities of air through perforated pipes.

The desired culture of yeast is carefully selected and carried in the laboratory. Special inocula are introduced into the pure culture system as needed.

The actual fermentation presents many problems, particularly as to the correct relationships between size of fermenters, dilution of the fermenting liquid, the regulation of the rate of wort additions, the pH to be maintained, the temperature, amount of aeration, length of the fermentation as well as the intricate process of growing the yeast from the single cell state through the pure culture fermenters and stock fermenters to the final end of the trade fermentation.

At the completion of the fermentation, the yeast must be concentrated and washed. Separators of special design are commonly used. The washed yeast is then stored until the drying procedure starts. Drum or spray drying may be used after which the yeast is milled if necessary and packed into suitable containers. Proper storage facilities are necessary to insure maintenance of quality.

Distillers' Dried Yeast

GUSTAVE T. REICH
1422 Chestnut Street
Philadelphia 2, Pennsylvania

Molasses distilleries can be considered potential producers of large quantities of dried yeast, either as a main or by-product. Three important steps must be considered in producing this yeast: 1) Removal of the largest possible percentage of alkaline earth compounds from the molasses because these tend to precipitate during fermentation and as a result contaminate the yeast to such an extent that the ash may be as high as 65 percent. 2) Recovery of the yeast before passing the beer to the beer still. 3) Removal of the gums and waxes from the yeast because these influence its quality and the ease of drying.

Process

The various steps are as follows: Molasses is diluted to a density of 40° to 45° Brix, heated to 180° F., and treated with sulfuric acid. The acid treatment serves three purposes. It hydrolyses the carbohydrates, maintains the proper pH, and converts the lime into calcium sulfate. In order to remove the highest percentage of calcium sulfate the type of crystal formation must be regulated by controlling the pH, temperature and time of reaction. A monoclinic crystal structure favors a more complete removal than does symmetric crystal structure. The dilution of molasses with water also effects the solubility of the calcium sulfate crystals.

The reaction between the lime and sulfuric acid is not rapid owing to the buffer action of various salts present in the molasses. Therefore the diluted molasses is held in a reaction tank at 180° F. for one hour at pH 3 to pH 3.5. From the reaction tank, the hot pasteurized liquor, containing the well-formed monoclinic crystals of calcium sulfate in suspension pass to the centrifugals. The calcium sulfate collected in the centrifugals is washed with water, while the clear effluent is collected, cooled to 80° F., diluted to 18° to 22° Brix, seeded with yeast and fermented.

Recovery and Treatment of Yeast

The beer is freed of its yeast and dealcoholized in the beer still when the fermentation is completed. The beer, containing 7 to 9.5 percent of alcohol by volume, is free of precipitated calcium sulfate. All the yeast present in suspension and that which has settled to the bottom of the fermenter during fermentation can be recovered.

The general procedure is to first pass the beer through a DeLaval separator. The partly concentrated yeast thus obtained is centrifuged a second time reducing it to 1/10 of its original volume. This slurry is washed with water and aerated in a specially designed flotation machine for the separation of gums and waxes. Conventional methods for air agitation were found lacking in efficiency. In order to get the best results various factors must be considered, namely density of the slurry, circulating load, contact period and volume of air used.

The yeast slurry containing 12 percent solids is dried on a double drum drier and then packed in a 3 ply paper bag. However, the yeast slurry may be concentrated first in an evaporator to a density of 30 percent solids and then dried.

The nutritive value of distillers' dried yeast is comparable to that of brewers' yeast as demonstrated in feeding experiments made at Pennsylvania State College. While its thiamine content is lower than that of brewers' yeast, its content of fat and some amino compounds is somewhat higher.

Two plants following the described procedure (patented and patents pending) produced 4,000,000 pounds of dried yeast annually. New plants are being erected in the Caribbean Islands where it is planned to use the yeast to combat malnutrition.

Food-Yeast Production from Wood-Processing Byproducts[1, 2]

ELWIN E. HARRIS
Forest Products Laboratory,[3] Forest Service
U. S. Department of Agriculture

Food yeast that is high in protein and vitamin and suitable for human or animal food may be produced rapidly and in good yields from the carbyhydrates contained in waste wood or those contained in byproducts from the chemical processing of wood.

Sugars from any wood source may, after the addition of inorganic nutrients, be used to grow yeast. Sulfite spent-pulping liquors, for example, contain sugars representing 9 to 11 percent of the weight of the wood. Logging and sawmill refuse, as well as wood discarded at woodworking plants, contain 45 to 70 percent of carbohydrates, which may be converted by hydrolysis to sugars in yields ranging from 35 to 65 percent of the weight of the wood. When wood chips are softened with steam for coarse fiber production, 10 to 30 percent of the wood is converted to water-soluble carbohydrates that may be converted to simple sugars suitable for yeast production by a secondary hydrolysis with dilute acid. Wood chips that are to be pulped by alkaline pulping processes may be given a short preliminary hydrolysis with dilute acid to convert 15 to 20 percent of the wood into soluble sugars without impairing its pulping quality. These sugars may be recovered by extraction and used for yeast production. Pentose and hexose sugars are equally suitable for use.

Most wood-sugar solutions produced by hydrolysis of wood contain substances inhibitory to growth of microorganisms, but yeast may be acclimatized to overcome this inhibiting effect. Because of this inhibiting property and the sterility of sugar solutions produced at high temperature in the presence of acid, it is possible to transfer yeast from a spent solution to fresh sugar solution repeatedly without contamination from the sugar source. This repeated transfer results in acclimatization of the yeast to conditions that favor rapid growth in the wood-sugar solution.

Experiments in batch production of yeast with conventional spargers for introduction of air showed that foaming is a serious problem, and large amounts of antifoam were required to control the foam. Because of the presence of inhibiting material, it was necessary to dilute the

---

[1] This report is a review of a Forest Products Laboratory address presented at a Yeasts in Feeding Symposium, Milwaukee, Wis., Nov. 8-10, 1948. Copies of the original address may be obtained from the Forest Products Laboratory, Madison, Wis., as Report No. D1754.

[2] Parts of the work described in this report were financed with funds supplied by the War Production Board and by the Research and Marketing Act.

[3] Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

wood-sugar solution for batch production. This decreased the amount of yeast that could be grown and increased the air requirement.

Because wood sugars are sterile, they are suitable for use in continuous propagation of yeast. For pilot-plant tests, a propagator was designed that utilized the foaming tendency of the liquid to obtain intimate contact of the media with air. Because sugar was fed continuously and yeast removed continuously, active growth of yeast took place at all times and the yeast was lighter in color than that obtained in batch operation.

In the system developed by the Forest Products Laboratory, air is dispersed mechanically through a rapidly rotating open-tube sparger that also acts as a pump to force circulation of the yeast and media through a central draft tube. This action prevents the accumulation of a nonbreaking foam on the top of the liquid. After removal of the yeast through an electronically-controlled valve in the bottom of the propagator, the foam is broken by addition of a small amount of antifoam and mild agitation.

In the pilot plant, maximum yields of yeast were obtained when approximately 200 cubic feet of air were used for each pound of dry yeast produced. A laboratory propagator of 40-gallon capacity was fed 20 liters of 5 percent sugar per hour, and air was introduced at the rate of 2½ cubic feet per minute. Twenty pounds of dried yeast were produced in a 24-hour day.

Nutrient requirements for yeast production were determined by varying each nutrient above and below the amount found suitable by preliminary tests. It was found that 3.4 pounds of nitrogen in the form of ammonia or urea, 1.6 pounds of P_2O_5 as phosphate salts or phosphoric acid, and 1.1 pounds of potassium sulfate or potassium chloride for each 100 pounds of sugar in the feed was necessary for optimum growth. The addition of organic nitrogen compounds or substances containing growth factors did not improve the yield or the rate of growth.

Sugar solutions in concentrations ranging from 0.5 to 8.0 percent were used for production of yeast. The ability of the propagator to control foaming when the amount of air necessary for high concentrations was used appeared to be a limiting factor. With high concentrations of sugar some alcohol was produced when amounts of air were used that could be controlled resulting in a lower yield of yeast.

Twenty strains of yeast, including strains of *Torula utilis, Torula utilis major, Torula utilis thermophilus, Candida albicans, Candida arborae, Mycotorula, Hansenula, Saccharomyces,* and some unidentified strains were tested. Many gave low yields on the first transfers, but after 12 to 15 transfers yields of yeast equalled 30 to 45 percent of the total sugar in solution. Several strains appear suitable, on the basis of yield and sugar utilization, for commercial production of wood-sugar solutions.

Wood-sugar solutions from various sources contain a mixture of sugars, sugar acids, and volatile organic acid that are utilized by yeast growth. Yields of yeast may at times be greater than the weight of the

sugar present because of growth on nonsugar products. Sugars from either hardwoods or softwoods are so completely used that only traces remain. Volatile organic acids are 75 to 85 percent utilized. Sulfite waste pulping liquor that also contains large amounts of lignin and sugar-decomposition products loses 50 percent or more of its reducing substances when used for yeast growth.

Yeast produced from wood sugars contains 46 to 56 percent protein, 2 to 7 percent fat, and 6 to 12 percent ash. Feeding tests indicate this yeast to be low in methionine but high in B-complex vitamins.

Producing Yeast from Processing Wastes[1]

W. D. RAMAGE AND J. H. THOMPSON
Western Regional Research Laboratory, Albany, California

Wastes from the canning of vegetables and fruits in the United States total about 4 million tons annually. Large amounts of potentially useful raw material are going to waste. Methods have been developed for converting some of the vegetable wastes to a high protein leaf meal, but the fruit processing wastes are still regarded mainly as a disposal problem. Consideration has been given to the production of yeast from fruit and vegetable processing wastes. Sugar from fruit-processing residues can be made available in juice at somewhere near one cent per pound as soon as a practical continuous press is developed, and sugar in citrus peel press liquor is available at even less cost. In the following discussion we shall consider only the direct application of sugar-bearing fruit and vegetable wastes in the growing of yeast.

Research workers in the Department of Agriculture have considered the use of processing residues for yeast production. The juice from asparagus butts and the juice from pear waste have been studied at the Western Regional Research Laboratory. The Southern Regional Research Laboratory and the U. S. Citrus Products Laboratory have studied liquor obtained during the manufacture of starch from sweet potatoes and citrus peel press liquor. The present discussion is concerned almost entirely with the results of this work, especially the pear waste studies.

In the case of juice from asparagus waste, no pilot plant studies have been made on the growing of yeast. This material has been considered to have greater value as a culture medium for other microbiological processes.

Two different types of continuous pilot plants for yeast production have been built and successfully operated—one using citrus peel press

[1] A more complete presentation of the material may be found in *Food Packer*, May, 1949, "Producing Yeast from Processing Wastes."

liquor and the other utilizing juice from pear cannery processing waste. Design and operating characteristics of the pear waste pilot plant are distinctly novel in some respects. The most noteworthy of these features are the use of mechanical agitation and mechanical air dispersion in the yeast propagator and automatic instrument control of the propagation at a constant pH. The rate of culture withdrawal is determined by the rate of wort feed to the propagator. The rate of wort feed is in turn controlled by a pH recorder-controller set to maintain a constant pH in the propagator. The yeast grows well at any pH between 3.5 and 6 and at temperatures in the range of 92 to 96° F. For practical operating reasons the pH is maintained at 4.5 and the culture temperature controlled at 94° F. Sugar concentration in the propagator must be controlled because the yeast becomes sluggish if too little sugar is present and sugar is wasted in the harvest stream if the sugar is added too rapidly. A sugar concentration of 0.1 to 0.2% is automatically maintained in the propagator by the control method used in this plant. Under the conditions of operation an air flow of 1.2 cu. ft. per minute per lb. of yeast in the propagator was found to be adequate when the air was well dispersed. Optimum air flow appeared to be in the range of 1.2 to 1.35 cu. ft. of air per minute per lb. of yeast in the propagator. Air flows below 1.0 cu. ft. were sharply limiting to both reproduction rate and yield. Air flows above 1.35 cu. ft. per minute per lb. of yeast in the propagator gave no improvement in rate or yield and tended to cause stratification of the yeast at the surface of the propagator. The air usage in this plant is small relative to yeast production and air usage in other plants.

The yeast output of the pilot plant is unusually large in proportion to the size of the propagator. Under good, steady-state operating conditions the yield of yeast was 57% of the weight of sugar fed and the reproduction rate of the yeast was 0.59 reproductions per hour. It is believed that the favorable results are due principally to two factors—the effective aeration supplied by the mechanical air dispersing system and the exact maintenance of constant operating conditions near the optimum point by means of instrument control. Such optimum conditions cannot be readily maintained with manual control or in batch propagations. A further important consideration is the high sugar concentration that can be used in the feed to the propagator without affecting yield. The sugar concentration of the feed did not appear to be yield limiting up to 0.64 lbs. of sugar per gallon of wort, which was the highest value used. The practical importance of these findings lies in the large production which can be obtained from a small yeast propagator with a minimum of operating supervision. The high reproduction rate results in a short average retention time for the yeast in the propagator and the high level of sugar concentration in the feed wort permits the maintenance of a high yeast cell-volume in the propagator. Maintenance of optimum growth conditions is relatively easy in a continuous system of this sort. Furthermore, a continuous, constant-volume system will produce more yeast for a given propagating volume than any combination of batch or semi-continuous propagators.

Taxonomy and Morphology of Yeasts

E. M. MRAK AND H. J. PHAFF [1]
Food Technology Division, University of California
Berkeley, California

The early investigations on yeasts were made prior to the use of pure culture methods. This resulted in a great many confusions, some of which still exist. In 1931 Dutch workers prepared the first of a series of recent monographs on the taxonomy of yeasts. The first was devoted to ascospore forming yeasts and the two subsequent volumes to the non-ascospore forming yeasts. Although this work has brought order out of chaos insofar as yeast taxonomy is concerned, the Dutch contributions should not be taken as the ultimate in yeast taxonomy. Laboratory procedures used in identification are being improved and several new species have been described since 1931. The systems used in these volumes are discussed.

Definitions of Yeast

Terms such as cultured, true, false, top, bottom, or others used to define yeasts according to their industrial behavior, have very little meaning from a botanical point of view. True yeasts are supposed to form ascospores, although this character is sometimes lost when they are held in culture.

Yeasts are commonly considered to be the organisms included in the sporogenous family *Endomycetaceae* and in the nonsporogenous families *Rhodotorulaceae* and *Torulopsidaceae*. The need for considering the families *Dipodascaceae* (including *Ascoidea*) and *Taphrinaceae*, as well as *Endomycetaceae*, with the yeasts has been demonstrated. It is likewise difficult to exclude such genera as *Ashbya*, *Eremothecium* and *Spermophthora*.

The term yeast should be broadened to include all organisms in the *Hemiascomycetes*, their imperfects, and possibly *Sporobolomyces*.

Characters Used In Classification

Families, subfamilies, and genera are based to a large extent on morphological characters. Physiological differences, on the other hand, play a more important role in the definition of species.

Gross Morphology: The occurrence and characteristics of pellicles (films, skins) are important factors in the differentiation of certain genera and species. Sediment formation as well as slant cultures and giant colonies are always described for each particular species, variety, or even strain. They are characterized as to glossiness, wetness, texture, form, surface morphology, edges, and color.

[1] The original paper with added illustrations may be purchased from Book Dept., ASUC Store or F. H. Stadtman, 339 Hilgard Hall, Univ. of California.

Morphology: The presence or absence of ascospores determines whether a specific yeast should be included in the "perfects" (ascospore-forming) or "imperfects" (nonascospore-forming). Characteristics of the sexual process such as the stage in the life cycle at which diploidization takes place, the formation of ascospores from diploid vegetative cells or fusing haploid vegetative cells, and the characteristics of asexual reproduction are important in generic differentiation. Morphological characteristics of the thallus are also commonly used for generic as well as species differentiation. Although most yeasts are unicellular, several are characterized by the formation of multicellular hyphae.

Cell shape, cell size and formation of thick-walled resistant spores are other morphological characteristics employed for classification.

Cytology: Cytological structures and characteristics are usually noted but seldom used for identification. The presence of carotenoid pigments is used to distinguish the family *Rhodotorulaceae.* Refractive granules, fat bodies, metachromatic granules and other cytological structures may be helpful in giving the taxonomist "leads," but are not employed as classification characters.

Physiology: Physiological characters play an important role in differentiation of yeasts, although they are seldom applied to the taxonomy of other fungi. Characteristics most commonly used are, fermentation of sugars, utilization of sugars, utilization of nitrogenous compounds and ability to grow in alcohol medium. Less important physiological characters are liquefaction of gelatin, ability to split fat, acid production and coagulation of litmus milk, acid production in ordinary media, and hydrolysis of aesculin or arbutin. Environmental characters such as temperature limits for growth and acid, sugar or salt tolerance are not considered very important in taxonomy and are seldom used.

Taxonomy of Ascosporogenous Yeasts

The yeasts are divided into sporulating *(Hemiascomycetes)* and nonsporulating forms *(Torulopsidaceae).* The *Hemiascomycetes* form typical ascospores by free-cell formation and are characterized by sexual cells and asci arising directly from the product of a sexual union. This group of organisms has been variously divided into orders and families. A convenient system involves separation into the three orders, *Dipodascales, Endomycetales* and *Taphrinales.* The first of these orders is easily characterized by the occurrence of plurinucleate gametangia and multispored asci. The last two, however, are quite difficult to separate on the basis of test-tube cultures. The order *Endomycetales* is generally considered to include those organisms more popularly known as the ascospore forming yeasts. They are generally uninucleate, form a limited number of spores per ascus and only in exceptional cases, are parasitic on plants. They do not form "pulcherrima cells." Members of the order *Taphrinales,* on the other hand, are all parasites on plants, producing characteristic erect asci on the surface of the host. Some species produce

"pulcherrima cells" in test-tube cultures. Various sub-families and genera are described and characterized in the original paper.

Taxonomy of Imperfect Yeasts

There is some evidence to support the view that this heterogeneous group of yeasts has arisen from those forming ascospores. The imperfect yeasts undoubtedly belong to the orders *Torulopsidales* or *Moniliales* in the *Fungi Imperfecti*. Only recently has Buchwold suggested a satisfactory arrangement for inclusion of the imperfect yeasts in the *Fungi Imperfecti*. The imperfect yeasts have been included in the two families *Torulopsidaceae* and *Rhodotorulaceae*. The family *Rhodotorulaceae* contains the single genus *Rhodotorula* characterized by the presence of carotenoid pigments and the absence of a pseudomycelium. The family *Torulopsidaceae* differs from *Rhodotorulaceae* in that carotenoid pigments are not produced. It is divided into the two subfamilies *Mycotoruloideae* which produces pseudomycelia, and *Torulopsidoideae* which does not produce pseudomycelia. Various subfamilies and genera are described and characterized in the original paper.

Other Organisms

Some provision should be made for imperfect forms of *Schizosaccharomyces*. If *Eremascus* and *Endomyces* are to be retained as yeasts, then the imperfect forms should be classified as imperfect yeasts. Inclusion of the genus *Geotrichum* with the imperfect yeasts would be helpful. The views of Diddens and Lodder and of Buchwold with respect to this are discussed in the original paper. The genus *Sporobolomyces* is not considered an Ascomycete and hence may not be included with the yeasts in the *Hemiascomycetes*. However, many of its characteristics are similar to those of yeasts and also, it is frequently isolated with yeast. In view of this it is important that the characteristics of *Sporobolomyces* be familiar to anyone studying yeast.

Ecology and Distribution of Yeasts

H. J. PHAFF AND E. M. MRAK
Food Technology Division, University of California
Berkeley, California

SUMMARY *

Numerous distribution studies have shown that yeasts are not as ubiquitous as bacteria, but display definite trends for certain habitats. Frequently, certain yeasts are only found on substrates of special chemical composition and concentration. Especially those distribution studies made with modern taxonomic methods are of great value for a better understanding of yeast ecology. Many of the yeasts described in older publications are so poorly described, that their identity in most cases is highly uncertain. A discussion will be given of yeast floras found on substrates high in sugar or salt content, or those which contain special carbohydrates, such as lactose, starch dextrins, wood, etc. Others are particularly adapted to the utilization of special nitrogen sources, such as nitrate, whereas others seem to be particularly suited to growth on proteinaceous substrates.

Unless special precautions are taken, ordinarily only the predominant organisms will be isolated by direct plating. It is more difficult to use selective media in the case of yeasts than bacteria, because of less variation in metabolism. Some success, however, has been obtained in isolating special yeasts by the use of selective media.

Yeasts do not only occur as spoilage organisms (Saprophytes) or as parasites in certain fungus diseases of animals and man, but some participate in special types of fermentations. The following yeasts are described and discussed: those occurring in substrates with a high sugar content such as in honey, on grapes, on sugared dried prunes and figs, and fresh fruits; those occurring on meat products; those occurring in brines; those associated with milk products; those associated with insects; plant pathogenic yeasts; those associated with deterioration of wood and wood pulp; yeasts from mushrooms and other fungi; those occurring in soil and ocean water; those involving special fermentation.

---

*Part of this paper has been published elsewhere. (Yeasts by E. M. Mrak and H. J. Phaff, *Ann. Rev. Microbial.* Vol. II, 1-46, 1948.)

Genetics and Hybridization in Saccharomyces*

CARL C. LINDEGREN
Southern Illinois University, Carbondale, Illinois

Segregation of Paired Hereditary Factors

Diploid yeast cells produce four-spored asci and in each ascus *paired* characteristics are segregated. When spore formation occurs a pair of genes controlling copulation segregates and the diploid cell (produced by mating of two different kinds of individuals) produces an ascus containing *two a* type spores and *two* alpha type spores. The fusion of these cells produces a diploid cell which develops into the standard large diploid cells and these in turn produce four-spored asci.

Recombination of Hereditary Factors

When the parents differ from each other in two pairs of character, four kinds of offspring are produced. Figure 1 shows the results obtained

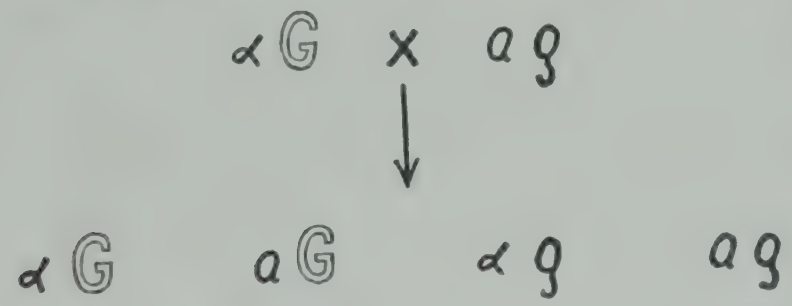

Fig. 1. The four kinds of progeny expected when an α G by ag mating is made.

when one parent is alpha mating type and a galactose fermenter (αG), and the other is an *a* mating type and a nonfermenter of galactose (ag). The progeny fall into four categories: (1) alpha mating type, galactose fermenter (αG); (2) alpha mating type, nonfermenter of galactose (αg); (3) *a* mating type, galactose fermenter (aG); and (4) *a* mating type, nonfermenter of galactose (*a*g). If the genes are not linked the four classes are produced in equal numbers. In the above case the four types of offspring are obtained in equal numbers—25 percent in each class. The ability to ferment various carbohydrates is under gene control, and cells can be produced containing any possible combination of genes. The abilities to ferment galactose, melibiose, maltose, sucrose

*This work has been supported by grants from Southern Illinois University, Anheuser-Busch, Inc., and the United States Public Health Service.

and alpha methyl glucoside are under the control of specific and different individual genes and various combinations of these types have been produced that result in new and unexpected combinations of fermentative abilities.

Galactose-Melibiose Fermentation

Hybrids between galactose-melibiose fermenters (G ME) and non-fermenters of these sugars (g me) were made. All four types of offspring were obtained (G ME, g me, G me, g ME). The G me cells were incapable of fermenting melibiose and produced gas in the presence of galactose. The g ME cells produced gas from melibiose by splitting the melibiose molecule into glucose and galactose, produced gas by fermentation of glucose, leaving a residual molecule of galactose.

Genetic Control of Raffinose Fermentation

Raffinose is a trisaccharide composed of fructose, glucose and galactose. *Saccharomyces carlsbergensis* is able to hydrolyze melibiose and ferments the entire raffinose molecule, leaving no residual melibiose. By hybridizing *S. chodati* and *S. carlsbergensis* we have developed pedigrees of yeasts differentiated by their ability to ferment melibiose and sucrose and have produced the four possible combinations of hybrid progeny (Table 1). The ability to ferment sucrose and raffinose correspond,

TABLE 1

Production of CO_2 When Different Sugars Are Used in the Fermentation Tubes

Genotype	Melibiose	Sucrose	Raffinose
ME SU	+	+	+
ME su	+	−	−
me SU	−	+	+
me su	−	−	−

proving that the ability of a yeast to produce gas from raffinose depends upon its ability to hydrolyze the sucrose moiety of the raffinose molecule. However, a yeast which is able to ferment melibiose but unable to ferment sucrose is incapable of breaking down raffinose. Therefore, raffinose cannot be split by the specific melibiase present in yeast. The complete fermentation of raffinose depends upon the action of sucrase, melibiase, and galactase.

Alpha Methyl Glucoside and Maltose

Maltose is an alpha glucoside of glucose; alpha methyl glucoside is a homologous compound in which the methyl radical occupies a similar position to that of glucose in the maltose molecule. Many chemists have assumed that because of the similarity in construction of the molecule, the same enzyme would split both maltose and alpha methyl glucoside. However, yeasts which are incapable of fermenting either alpha methyl

glucoside or maltose, when mated to yeasts capable of fermenting both of the sugars, produced the expected four kinds of offspring, showing that there is no interaction between these two enzymes. Isolation of crude enzyme preparations supports the view that two different enzymes are involved; one specifically capable of acting on alpha methyl glucoside, and the other specifically capable of acting on maltose. Subsequent work in this laboratory has shown that the number of yeast carbohydrases is larger and their substrate specificity much narrower than has hitherto been supposed. Table 2 shows the eight different genotypes produced

TABLE 2

Different Yeast Genotypes Obtained from Heterozygous Hybrids

MG	MA	SU	MG	ma	su	mg	ma	SU
MG	MA	su	mg	MA	SU	mg	ma	su
MG	ma	SU	mg	MA	su			

by mating a fermenter of alpha methyl glucoside, maltose and sucrose by a nonfermenter of the three carbohydrates. Each culture is capable of fermenting only the sugars indicated by the capital letters, and incapable of fermenting sugars indicated by the small letters. This reveals that the three sugars are acted upon by specifically different enzymes in spite of the fact that each is an alpha glucoside.

Nutritional Deficiencies In Yeasts

The large variety of freely interbreeding species of *Saccharomyces* has also provided a source of genes differentiating yeasts with regard to the synthesis of some B-vitamins. Ultra violet radiation is another major source of mutants. Individual haploid cultures dependent on lysine, leucine, tryptophane, histidine, adenine, arginine, and uracil have been obtained. Each mutant culture has been mated to a normal strain and the hybrid analyzed genetically for regular segregation.

The synthetic cycles in yeast approximate those in *Neurospora* and higher organisms: the tryptophane deficiency may be satisfied either by anthranilic acid or indole; the uracilless mutant is satisfied by uridine and less well by uridylic acid; the arginine dependent culture grows slowly with added ornithine and less well with added citrulline. The histidineless mutant is satisfied by the L-form, one-half as well by the DL-form and not at all by the D-form.

Gene Conversion

Numerous cases of irregular or non-Mendelian inheritance have been encountered. This phenomenon results from the transfer of hereditary material from one gene to another. Conclusive proof of this view was achieved by the tetrad analysis of hybrids in which the longest chromosome was heterozygous for six loci. Non-Mendelian segregations of the AD/ad locus did not coincide with similar segregations at the other loci

in the same chromosome. This phenomenon has been interpreted to be due to conversion of one gene into its allele by contact in the heterozygous condition and has opened up new possibilities in fundamental genetical theory which are discussed in my new book "The Yeast Cell." [1]

[1] Lindegren, Carl C. *The Yeast Cell,* 384 pp., illus., Educational Publishers, Inc., St. Louis (1949).

Inheritance of Enzymatic Characters in Yeast

CATHERINE ROBERTS
Carlsberg Laboratorium
Copenhagen, Valby, Denmark

A study of the maltose and galactose fermenting ability of the progeny of a hybrid between *Saccharomyces cerevisiae* and *S. chevalieri,* as well as of several backcross hybrids to the recessive *S. chevalieri,* revealed that *S. cerevisiae* possesses 3 polymeric genes of varying intensities responsible for maltase production (M_1, M_2, M_3) and one gene (G) for galactozymase production. Yeast types containing M_3 and g_s ferment the respective sugars only after a long-term adaptation period in the presence of the sugar, involving a gradual increase in enzyme production until a sufficiently high concentration is reached to bring about visible fermentation. Studies of the interspecific hybrids *S. chevalieri* x *S. italicus* and *S. cerevisiae* x *S. italicus* made possible the erection of the following formulae for these 3 species with respect to the production of maltase (M), galactozymase (G), and the enzyme responsible for the fermentation of raffinose and sucrose (S):

S. cerevisiae $M_1\ M_2\ M_3\ G\ S_1\ S_2\ s_3$
S. chevalieri $m_1\ m_2\ m_3\ g_s\ S_1\ S_2\ S_3$
S. italicus $M_1\ m_2\ m_3\ G\ s_s\ s_2\ s_3$

Evidence for the existence of linkage between the maltase gene and one of the 3 polymeric saccharase genes was obtained from the hybrid, *S. italicus* x *S. chevalieri.* These experimental results can be explained according to the principles of Mendelian inheritance without recourse to Lindegren's cytogene theory.

The Experimental Control of Enzymatic Constitution in Yeast

S. SPIEGELMAN
University of Illinois, Department of Bacteriology,
362 Noyes Laboratory of Chemistry, Urbana

The primary purpose of the paper is to present results of a research program which has had as its major aim the delineation of the factors which determine the physiological and biochemical characteristics of yeast cultures. The effect of genes and the type of control exerted by them in determining the character of the enzymes found in the cell was studied by the phenomenon of enzymatic adaptation in yeast cells.

Enzymatic Adaptation

It was established that a change in the pattern of enzyme activity can be obtained in cells in which there is *no change of genetic constitution.* This conclusion is made possible by the fact that the appearance of enzyme can be observed in these cultures in the absence of cell division. Natural selection of mutant types can not therefore be invoked to explain the changed physiological characteristic of the population.

Fractionation of adapted and unadapted cell preparations revealed that the apoenzyme or protein content of the enzyme complex is modified during the course of the adaptation. That only this modification is involved follows from the ability of unadapted cofactor preparations to replace equivalently similar preparations derived from adapted cells in the activation of adapted apoenzyme to galactose fermentation.

These results imply that the mechanism of gene control over enzymatic constitution far from being rigid, is extremely flexible and that the statement "genes control enzymes" does not describe the actual situation, but it should be rephrased "genes determine the *potentiality* for enzyme formation." Whether or not the enzyme is *actually* formed in the cytoplasm is apparently mediated by other factors of which the presence of substrate is obviously one. The kinetics of enzyme formation is autocatalytic in nature.

The protein source employed for the formation of enzyme may be either an external source of nitrogen if it is present or in its absence the cell will use existing enzymes as a source of protein to form the enzyme being induced by substrate. The severity of this type of competitive interaction varies considerably and extends over a range which includes complete absence of effect for certain enzyme pairs to complete suppression of one in other instances. It is interesting and important to note that examples of such competitive interaction between enzyme forming systems are not confined to the yeasts. Actually the first instances of such phenomena were observed in bacteria.

The existence of competitive interactions among enzyme forming systems would appear to preclude any hypothesis which involves a

transformation of protein molecules from inactive units which are already so highly specific that their potential activities are already rigidly determined. On this basis it seems reasonable to eliminate the pepsinogen to pepsin type of conversion as a plausible model for enzymatic adaptation or its kinetics.

Genes and substrates can function as controlling elements either via their capacity to influence the molecular stability of the enzyme molecules, or their ability to modify the conditions under which competition amongst the enzyme forming systems is taking place. Evidently, then the same agents or conditions (absence of synthesis which effectively inhibit the formation of new enzymes can under certain conditions also effectively prevent the loss of any existent enzymatic capacities. The property of molecular stability plays a relatively minor role in the mechanisms controlling the maintenance of the enzyme patterns studied in these particular strains. Apparently, the extent, severity, and kind of competition does, however, determine the types and amounts of enzymes found in the cytoplasm of the cell. Whatever the generality of these results may be, it is nevertheless true that any theories of gene action or substrate action which seek to explain how these factors can control enzymatic constitution must provide a mechanism whereby these agents can perform their function by influencing the outcome of the competitive interactions amongst the enzyme forming systems of the cell.

Synthesizing systems would compete not only for material, but also for the energy necessary to their function. It has been suggested that the phosphate containing proteins, the nucleoproteins, are the primary agents involved in providing the energy required for protein synthesis in general and enzyme synthesis in particular.

The work cited led to a concept of gene-enzyme relationship which may be summarized as follows. Genes produce at various rates more or less complete replicas of themselves which enter the cytoplasm. These replicas or *plasmagenes* are nucleoprotein in nature and possess to varying degrees the capacity of self-duplication. Their presence in the cytoplasm controls the types and amounts of proteins and enzymes synthesized. These plasmagenes, like all self-duplicating entities, compete with each other for material and energy and the outcome of such competitive interactions then determines the enzymatic constitution of cytoplasm.

Inherent in this concept is the possibility of changing the ultimate result of this competition by varying the conditions (for example, substrates available) under which it takes place. It also provides the kind of flexibility in the control mechanism of gene over enzyme synthesis required to explain the primary fact underlying the phenomenon of enzymatic adaptation, viz., possession by a cell of a particular gene in the nucleus does not guarantee that the corresponding enzyme will be found in the cytoplasm. Under any given set of conditions, only those genes, which produce plasmagenes capable of competing successfully for protein will express their potentiality for determining the enzyme pattern.

The validity of certain predictions based on this theory have been tested experimentally and are discussed in the original papers.

Nutrient Requirements of Yeast

M. A. JOSLYN
Division of Food Technology, University of California
Berkeley, California

Although the general growth requirements of yeast have been investigated, a chemically defined medium on which yields comparable to those obtained on natural media has not been prepared. Systematic investigation of the growth requirements of yeast which was stimulated by investigation of the bios needs is still to be made. Studies with synthetic media have been limited to the evaluation of the ability of yeasts to produce particular cell constituents, in determination of vitamin requirements of various yeasts and more recently for the purposes of classification. An adequate synthetic medium can be defined only in general terms on the basis of our present knowledge of carbon assimilation, nitrogen assimilation, mineral metabolism, and growth factor requirements. The optimum pH, temperature, oxygen or E_h requirements are known for but a few species.

Carbon Requirements

The carbon requirements can be satisfied by glucose or sucrose for most yeasts; the utilization of other carbohydrates is still in a rather confusing state, particularly with regard to the disaccharides. Among the hexoses, differences exist in their ability to support growth even in the biologically similar glucose, fructose, and mannose. While most yeasts will ferment glucose more rapidly than fructose, the so-called "Sauternes" yeast will ferment fructose more rapidly. Some species of yeast will ferment galactose after previous adaptation; other species do not utilize it. The pentoses, arabinose and xylose, are usually not utilized by most yeasts, although they are readily assimilated by many molds and certain bacteria. Many organic compounds other than sugars can be used as sources of carbon, and some of these are of interest in stimulating certain metabolic activities as well as being useful in classification.

Nitrogen Requirements

The nitrogen requirements of yeast in general can be supplied by ammonium salts, urea or organic nitrogen compounds. Asparagine, aspartic acid, glutamic acid, ammonium phosphate, leucine and arginine are also good sources of nitrogen for *Saccharomyces* species. For the top fermentation brewers' yeast, ammonium phosphate is superior as a nitrogen source for both fermentation and growth. Certain amino acids which alone were either ineffective or had toxic effects on growth had beneficial effects when used collectively. Among the organic nitrogenous compounds which were found to have stimulating effects on growth of yeast in a synthetic medium may be mentioned asparagine, aspartic acid and leucine.

Growth Factors

Inositol, thiamine, biotin, pantothenic acid, and pyridoxine have been shown to be required by many yeasts. Other compounds such as nicotinic acid, para amino benzoic acid, and various pyrimidine and purine compounds have been shown to be stimulatory to some yeasts. The relative concentrations and kinds of nutrilites to be added, however, is still in a contradictory state. Yeasts during a long incubation period show considerable ability to grow in the absence of various nutrilites and under such conditions one nutrilite alone may serve, whereas for rapid growth several may be required.

In addition to inositol, thiamine, biotin, pantothenic acid, and pyridoxine, other growth factors were suspected on the basis of the finding that liver and yeast extracts promote more rapid growth when added to a medium containing all five nutrilites. A higher concentration of the known nutrilites was not found to produce an equivalent stimulating effect. It was found, however, that for one strain of bakers' yeast, biotin, calcium pantothenate, inositol, pyridoxine and thiamine supplied all the growth factor requirements. No additional factor in corn steep liquor or malt sprouts was found necessary to obtain high yields under conditions of optimum aeration.

Mineral Requirements

Investigations of the mineral requirements that have been made are for the most part inadequate; knowledge of the requirements of trace elements is in a particularly controversial and confusing state. Copper, iron, magnesium, potassium, phosphorous and sulfur have been shown to be required. The requirement for other minerals, particularly the micro-nutrient elements, has not been conclusively established. Knowledge concerning the mineral requirement of yeast has been based on the composition of yeast ash, on investigations of bios requirements in which nutrient solutions containing mineral salts and sugar were used in growth studies, on investigations of toxic and antagonistic effects of salts, and more recently on the metabolism of the more important cellular constituents. Aspects of the phosphorous, sulfur, iron, copper, potassium, calcium and magnesium metabolism, have been investigated.

The requirement for inorganic phosphate and for potassium is well recognized, but there are still differences of opinion as to the concentration of potassium phosphate which will meet these requirements. The chemistry of phosphate metabolism has been well established for *S. cerevisiae*. The conditions for phosphate uptake and the transformations which it undergoes in the metabolism of carbohydrates are fairly well understood. The role of phosphate in fat metabolism and in protein metabolism has yet to be established, and the possible role of phosphates in regulating permeability, moisture relations, and pH within the cell, are unknown.

Potassium, it is believed, specifically stimulates the reaction of phosphopyruvic acid with adenylphosphates, but this is still in a contradictory

state. Magnesium is known to function in carbohydrate metabolism as an activator of at least one transphosphorylase, but the function of calcium is unknown. The requirements for sulfur, iron, and copper are based upon strong physiological grounds. The requirements of trace elements, however, are still largely unknown. Boron, copper, iodine, iron, zinc, manganese, molybdenum and thallium, have at various times been shown to promote growth.

Agitation and Aeration

Although the role of mechanical agitation and of aeration in the nutrition of yeast is recognized, it is still largely unexplained. Very few systematic studies of these factors have been made. Mechanical stimulation of budding and the removal of excess of carbon dioxide have been suggested as possible explanations. The maintenance of a desirable oxidation-reduction potential is another possibility. Although the oxidation-reduction potentials of metabolizing yeast suspensions have been measured, and it has been shown that the O-R potential is characteristic of the type of metabolic activity, systematic investigations of this aspect of nutrition have not been made.

The Role of Vitamins in the Life of Yeasts

PAUL R. BURKHOLDER
Yale University, New Haven, Connecticut

Special substances which are known to be required for growth of microorganisms are also important in the nutrition and metabolism of higher plants and animals. It is now believed that the requirements of certain microorganisms for specific nutritional factors reflects the evolutionary loss of their ability to synthesize these compounds which are known generally to be fundamental constituents of protoplasm.

Bios and Growth Factors

The role of vitamins in the life of yeast was recognized as a result of the failure of a small inoculum of yeast to grow normally upon a simple medium unless a little organic matter extracted from yeast was added to the nutrient solution. This "life-giving" substance was called "bios." After demonstration of the multiple nature of bios, it became apparent that proper evaluation of bios and vitamins in yeasts could be made only when the identity of the components of these complexes became known. Forty years after the advent of the bios concept into microbial physiology it was concluded that five known compounds, inositol, thiamine, biotin, pantothenic acid, and pyridoxine, are important growth factors for this

group of plants. During these four decades, knowledge of growth stimulants for yeasts and other microorganisms developed hand in hand with discoveries in the chemistry and biology of the vitamins required as accessory food factors by animals. Sometimes the important first discoveries were made with yeasts or bacteria, as with inositol, biotin and pantothenic acid. In other instances, the lead was taken in the vitamin field, as in the studies of thiamine and pyridoxine in animal nutrition. Always, however, there was a general tendency toward the merging of microbial growth factors and animal vitamins. Emphasis in this first period was placed primarily upon the isolation, identification and chemical synthesis of growth-promoting substances.

In the early studies on growth factors of yeasts, many difficulties were encountered because of a lack of appreciation for the role of inheritance in determining the ability of any species or variety to synthesize its own vitamins out of elementary constituents of the medium. In determining the exogenous needs of different yeasts, the composition of the basal medium is also of very great importance.

The vitamin requirements of numerous species of yeasts belonging in many genera have been studied. A summary of the B vitamin requirements of 163 strains belonging in 110 named species and varieties showed that many species and strains are capable of synthesizing their own B vitamins, while numerous other yeasts require an exogenous supply of one or more vitamins in the nutrient medium for optimum growth to occur. Thus 86 named kinds were heterotrophic for one or more vitamins. Deficiencies occurred as follows: biotin 78, thiamine 33, pantothenic acid 30, inositol 15, niacin 13, and pyridoxine 13. No deficiency for riboflavin has yet been observed in yeasts, although this is a common requirement among certain groups of bacteria. Examples of yeasts showing no outstanding vitamin deficiency are the following: *Hansenula anomala, Pichia membranaefaciens, Torulopsis utilis* and *Zygopichia chevalieri.* Some species were found to be deficient for as many as 6 vitamins, i.e., *Hanseniaspora melligeri, Kloeckera africana, K. brevis,* and *K. linderi.* Between these extremes many single and multiple deficiencies occur.

It has been stated that vitamins A. D, E, K and C have no known function in the metabolism of yeast. Carotenoids and ergosterol are produced by yeasts, but their functions in the metabolism of these plants are unknown. It has been suggested that the apparent absence of ascorbic acid from yeasts may be taken as an indication that it belongs in a separate category from the other vitamins.

Detailed discussion of the role of inositol, biotin, thiamine, pantothenic acid, niacin, B_6 group, B_{12} and other factors in yeast metabolism is presented in the original paper.

Classification

In any satisfactory schema of classification of microorganisms, consideration must be given to morphological and physiological characters

which are reasonably stable and recognizable. As in bacteriology, so in zymology taxonomic workers have utilized the growth responses of their organisms to different carbohydrates, various nitrogen compounds, etc., in order to obtain information complementary to the characters of morphology observed in vegetative growth and reproductive differentiation. With the advent of our knowledge concerning specific growth factor requirements of yeasts, it has been proposed that these substances be employed in the classification and nomenclature of these plants. The literature dealing with classification is reviewed in the original paper.

Genetics

The inheritance of morphological and biochemical characteristics of yeasts has been sufficiently studied to indicate clearly that the principles of genetics operate in these plants. Studies on the phenomena of enzyme adaptation and the development of new techniques for making controlled genetic crosses in mating types of yeasts have resulted in valuable contributions to science. The application of methods which have been successful for inducing mutation in *Neurospora* and bacteria have only been initiated recently in zymology. Only a few investigations dealing with the inheritance of vitamins in yeasts can be reported from the literature, but research is progressing in this field. Perhaps the principal "bottleneck" in yeast genetics is concerned with the tedious task of isolating the ascospores by microdissection. A program of biochemical genetics in yeasts should provide important new information concerning pathways of biosynthesis and mechanisms of inheritance in organisms which have already contributed their share to the advancement of knowledge and human welfare. The literature dealing with genetic studies influencing nutrient requirements is reviewed.

Microbiological Assays

The development of microbiological assays for vitamins of the B group naturally followed upon discovery of the quantitative response of yeasts to increments of growth factors added to suitable basal media. The methods of detecting graded responses of yeasts depend upon turbidimetry of growth or the estimation of CO_2 evolved in anaerobic fermentation. Important requisites for a satisfactory assay include extreme accuracy in execution of all manipulations, knowledge concerning specificity and range of the response, suitable preparation of the sample, proof of quantitative recovery of growth factor added to natural samples, and lack of drift in the results obtained with unknowns at different levels. The various assays in which yeast have been employed are given.

New Methods in Taxonomic Study and Maintenance of Yeasts[1]

LYNFERD J. WICKERHAM, KERMIT A. BURTON, AND RICHARD J. GILL
Northern Regional Research Laboratory [2]
Peoria, Illinois

The number of characteristics used in the past for the classification of yeasts has been so small that when variation has occurred in one of the characteristics, evaluation of the significance of the variation often has been difficult. The taxonomist has had little choice other than guess whether the variation was of sufficient magnitude to set related strains apart as separate species, as varieties, or as somewhat dissimilar strains of the same species.

The authors have increased the number of tests for use in the classification of yeasts so that the differences are multiplied in those cases which are matters of differences between species, and the similarities are multiplied in those cases which are merely differences between strains of the same species. Thus, opinions regarding speciation have been made appreciably less subjective.

The principal techniques used by the authors are mentioned in the following paragraphs. Most of the procedures involve the use of chemically defined media which have been made available for general use.[3]

1. The ability to form ascospores is determined by the use of carrot wedges, malt extract agar, vegetable juice agar, and malt extract-yeast extract agar.

2. Morphological characteristics, especially the ability to produce hyphae, are determined by the dalmau plate technique. Yeast morphology agar, a chemically defined medium, is used. It consists of pure B-vitamins and trace elements as well as salts, and carbon and nitrogen sources. The yeast grows both aerobically and anaerobically in a single plate preparation. The anaerobic growth is developed underneath a sterile coverglass, thus permitting the direct use of high power objectives to study the natural arrangement of hyphae and the blastospores which they bear.

3. Fermentation tests show whether a yeast can produce gas from glucose, galactose, maltose, sucrose, lactose, melibiose, or raffinose.

---

[1] Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946. A more detailed report of this study is to be published in a government Technical Bulletin, USDA.

[2] One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Supplied by the Difco Company.

Because many yeasts ferment no sugars at all, this technique is of somewhat limited use.

4. Carbon assimilation tests, in which some 40 carbon-containing compounds are employed, indicate which sources of carbon various species can use for growth. Ten or twelve of the most useful compounds are then selected for separating the species of a genus. The basal medium is chemically defined and complete except for the carbon source. The powdered medium is dissolved in distilled water with an amount of the test compound that produces a tenfold concentrate. It is sterilized by passage through a Seitz filter, stored in a refrigerator, and, as needed, 0.5 ml. amounts are added to 16 mm. test tubes, each containing 4.5 ml. of sterile water.

A set of media containing the various carbon compounds as well as the medium containing nitrate and the vitamin-free test medium described subsequently, are inoculated very lightly with a suspension of yeast cells diluted to a definite density. The cultures are incubated at 25° C., being read generally at 6 to 7 days and again at 20 to 24 days. Density of growth is determined after shaking the tubes, by placing them against a white card on which India ink lines are drawn about $\frac{1}{4}$ of a millimeter wide. If growth completely obscures the lines, the density is recorded as 3+. If the lines appear as somewhat dark broad bands, the density is recorded as 2+. If the lines are distinguishable as such, but the edges are indistinct, the reading is recorded as 1+; and no growth is recorded as minus. Three-plus and 2+ readings are considered as positive, and 1+ readings are considered as weak. The readings made at 7 days reveal which of the carbon compounds are slowly assimilated by means of adaptive reactions.

5. Nitrogen assimilation tests are limited to the use of nitrate. Potassium nitrate is added to a chemically defined medium which is complete except for a source of nitrogen. Most yeasts show moderate to heavy growth after 7 days of incubation in this medium even though they cannot assimilate nitrate. Growth is made possible for nitrate-negative yeasts partly by the excretion of assimilable nitrogen compounds from the cells of the inoculum and partly by the small amount of ammonium sulfate carried over with the medium in which the inoculum is grown. After 7 days of incubation, one loop of the culture is transferred to a second tube of nitrate medium. If the second tube shows a 2+ or 3+ amount of growth after 7 days of incubation, the yeast is capable of assimilating nitrate.

6. The ability of a yeast to grow in the absence of vitamins is determined in a chemically defined medium which is complete except that it contains no vitamins. Because the cells of the inoculum may contain stored vitamins, the first tube may show heavy growth after 7 days of incubation, even though the yeast is dependent upon its environment for one or more vitamins. Therefore, when the first culture is 7 days old, one loop is transferred to a second tube of vitamin-free medium. If the

second tube shows a 2+ or 3+ amount of growth after 7 days, the yeast under test does not require an external source of vitamins.

The vitamin-free base may also be used for identification of the individual vitamins required by yeasts.

The second part of the paper is concerned with the preservation of yeasts. Two procedures are used. The usual stab cultures are prepared every 4 months for all strains which are used frequently. The cultures are stored at about 5° C. between transferrings. The medium used for stock cultures and for general cultivation is yeast extract-malt extract agar. It consists of 3 grams powdered yeast extract, 3 grams powdered malt extract, 5 grams peptone, 10 grams glucose, and 20 grams agar per liter of distilled water. The pH is not adjusted.

All yeasts are preserved by the lyophil process. To preserve them, the cultures are grown on slants, the growth is suspended in a small volume of sterile blood serum, and the suspension is placed in a sterile glass tube to a depth of approximately 2 mm. The suspension is frozen at approximately —40° C. Then the temperature of the bath is raised to —5° by adding to the bath solvent which has been at room temperature. When the cells are visibly dry the tubes are raised above the freezing bath. The vacuum pump is allowed to run an additional 30 minutes while the tubes are at room temperature, and during the ensuing period while the tubes are being sealed off by a crossfire torch. The lyophilized yeasts are stored in a refrigerator.

CFTRI-MYSORE
2467
Yeasts in feedin..

— 30.11.73 29/11

— 24.7.74 23.7.74